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U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF SOILS—BULLETIN No. 73.
MILTON WHITNEY, Chief.

STUDIES IN SOIL OXIDATION.

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OSWALD SCHREINER AND M. X. SULLIVAN,

ASSISTED BY F. R. REID.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Soils,
Washington, D. C., July 22, 1910.

Sir: I have the honor to transmit herewith the manuscript of a scientific paper entitled "Studies in Soil Oxidation," by Dr. Oswald Schreiner, Dr. M. X. Sullivan, and Mr. F. R. Reid, of this Bureau. This article embodies the results of work carried out in this Bureau and is an important contribution on some little understood factors in soil fertility. The mild but effective oxidation shown to exist in soils has a very appreciable effect in aiding the decomposition of organic matter in the soil. The importance of the organic constituents of the soil makes these results especially interesting and the material throws additional light upon the question of soil fertility, and I have, therefore, the honor to recommend that this material be published as Bulletin No. 73 of the Bureau of Soils.

Respectfully,

Milton Whitney,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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STUDIES IN SOIL OXIDATION.

INTRODUCTION.

The prevailing ideas in regard to the maintenance of soil fertility have been largely based on the views brought forth by Liebig, that each crop requires certain elements from the soil and that the crops will not flourish where the appropriate elements are lacking or are present in insufficient quantities. Every soil, according to Liebig, contains some element in the minimum. Whatever element this minimum may be, it determines the abundance and continuity of the crop. The only fertilizer which acts favorably is that which supplies a deficiency of one or more of the food elements in the soil. In common practice, fertilizer addition to soils has resolved itself into supplying the soil with nitrogen, potassium, phosphorus, and sometimes lime, not necessarily because the soil always is lacking in these elements, but on the ground that they are in a form not available to plants. The practical application of fertilizers is founded on the restitution of the four elements among the eighteen or more elements considered as playing a part in agriculture. The other elements are not replaced for the reason that some, like carbon, hydrogen, and oxygen, are abundant in the assimilable form of water, free atmospheric oxygen, and carbon dioxide; that some, found among the fixed constituents of plants and in the plant ash, such as iron, calcium, magnesium, sodium, sulphur, chlorine, and silica, exist in the soil in such quantities that they can not be exhausted, and that others, though found in the vegetable ash, appear in such small quantities, if they appear at all, that it is doubtful if they are essential. These elements are manganese, titanium, copper, zinc, barium, iodine, and fluorine.

The theory of mineral requirements assumes that the action of fertilizers is on the plant rather than on the soil, regards the soil as a trough from which plants may receive their food supply, and, moreover, takes into account only the higher plants. But the soil, instead of being an inert reservoir, is the seat of physical, chemical, and vital actions which directly or indirectly influence soil fertility. The roots of plants and plant débris, with their biochemical activities, microorganisms, worms, enzymes from various sources, catalytic action of organic and inorganic matter of the soil, the interrelation between the various activities and the effect of fertilizers upon them, and the processes going on in humus, must be considered in any thorough

study of soil fertility. The roots of higher plants are not simply absorbing organs, but possess the power, through oxidation, reduction, and otherwise, to make changes in the soil ingredients—changes which are undoubtedly modified by various compounds, organic and inorganic. Besides serving as nutrients for plants, the fertilizing salts commonly used and the soil constituents are undoubtedly all doing work in the soil, modifying the various reactions in a complex, ever varying medium, and, in particular, modifying the kind, the number, and the activities of the microorganisms which are present in vast numbers, especially in cultivated soils, associated with the organic matter and undoubtedly exercising important functions, good or bad, in connection with the growth of the higher plants. It is only by a study of the chemical, physical, and biological activities going on in the soil that we can get a thorough insight into the problems of soil fertility. The physical and chemical sides have been studied extensively, the biochemical side but little. Whatever adds to our biochemical knowledge of soils advances and broadens our understanding of the complex problem of soil fertility. Accordingly, the prominent processes of oxidation and reduction in soil and by roots growing in the soil have been made the subject of considerable study. Some of the results and deductions obtained are given in the following pages.

BIOLOGICAL OXIDATIONS.

The literature dealing with the oxidizing power of plant juices is very voluminous. Within recent years our knowledge of the processes going on within the plant have been greatly enlarged by the studies which have been made on the oxidizing enzymes. This work has been summarized by Czapek[a] and by Bach,[b] while more recently Kastle[c] has presented in a very comprehensive way the subject of oxidases and oxygen catalysts concerned in biological oxidations. The literature is still growing apace. The power of the plant roots to bring about extracellular oxidation, especially of organic material in the medium in which the plant root is growing, whether this be soil or solution, has, on the other hand, received less attention, although from the standpoint of soil fertility this oxidizing power is not only highly interesting, but of the greatest importance.

Molisch[d] appears to have been the first to demonstrate the oxidizing power of intact roots. He found that roots were capable of oxidizing various organic substances, such as guaicol, pyrogallol, and gallic

[a] Biochem. der Pflanzen, Vol. **ii**, 368 (1905).

[b] Monit. Sci., **64, 65**, 321, 549 (1906).

[c] Bul. 59, Hygienic Laboratory, Public Health and Marine-Hospital Service of the United States Treasury Department (1910).

[d] Sitzungsber. Akad. Wiss. Wien. Math. nat. Kl., **96**, 84 (1887).

acid. Czapek[a] repeated some of the investigations made by Molisch and raised some doubts as to the correctness of Molisch's conclusions. The fact that roots have oxidizing powers as set forth by Molisch has been corroborated, however, by the investigations of Raciborski,[b] who used a number of reagents and widely different plants. He did not find a Phanerogam the roots of which did not have the property of extracellular oxidation. Great differences, however, existed between the oxidative power of different plants.

Schreiner and Reed[c] more recently studied the relation between the oxidizing power of wheat roots and soil fertility. They found that: (1) The oxidizing power of plants grown in extracts of productive soils was greater than that of plants grown in extracts of unproductive soils; (2) treating the soil extracts with an absorbing agent was usually beneficial to oxidation; (3) the distillate of a poor soil extract which contains volatile toxic compounds was less favorable to oxidation than the residue remaining after distillation; (4) the presence of added toxic organic substances in solution was deleterious to the oxidizing power of the plant roots; (5) the oxidizing power of the plant, especially in the presence of nitrates or lime, was able to lessen the toxicity of such solutions; (6) calcium salts and phosphates also increased the oxidizing power of the wheat roots, while potassium salts tended to retard oxidation.

The work of Schreiner and Reed showed clearly that oxidation by plant roots is a factor which has considerable agricultural interest, especially from the viewpoint that such oxidation is able to change the organic matter in the medium in which the plant is growing and that processes promoting oxidation play a large part in the best methods of soil cultivation, tillage, and crop rotation.

Further work on the extracellular activities of growing roots has brought out clearly the fact that the biochemical action of roots on the constituents of the medium in which they are growing must be considered in soil fertility problems, and accordingly the power of the root to bring about reduction processes, so often the accompaniment of oxidation, has been given some attention.

REDUCTION BY ROOTS.

As has been stated previously, the roots of many plants, such as wheat, have an oxidizing power.[d] This property is readily shown by certain chromogens (alphanaphthylamine, benzidine, phenolphthalin, aloin, guaiac, pyrogallol, etc.). When chromogens like alphanaph-

[a] Jahrb. wiss. Bot., **29**, 321 (1896).

[b] Bul. Acad. Sci., Cracovie, 1905, 338, 668, 693.

[c] Bot. Gaz., **47**, 355 (1909); Bul. 56, Bureau of Soils, U. S. Dept. Agr.

[d] For work pertaining to root oxidation, see Schreiner and Reed, The Rôle of Oxidation in Soil Fertility, Bul. 56, Bureau of Soils, U. S. Dept. Agr.

thylamine and benzidine are used, the colors due to oxidation are shown on the root itself. The most marked oxidation is shown by a narrow but very distinct band of color just back of the root cap. Then comes a practically colorless zone and then a broad colored zone, the color becoming less intense toward the upper part of the root.

In regard to the practically colorless zone, or the zone with little color just back of the region of intense coloration, it seemed as if the lack of color might be due to a reducing power, a reductase, or possibly an antienzyme.

The possibility of an antienzyme is suggested by the work of Bertel,[a] who showed that with the exclusion of oxygen there accumulated in the cells of the lupine roots larger quantities of tyrosine than are usually found. The tyrosine which arises from the protein degradation in the early stages of the seedling's growth is normally oxidized by the enzyme tyrosinase to homogentisic acid and oxidation products of the latter. With the exclusion of oxygen, this oxidation is retarded and tyrosine accumulates.

Subsequently Czapek[b] showed that "a short time after the beginning of geotropic induction there appears a retardation of the normal destruction of tyrosine to be recognized by an accumulation of homogentisic acid." The cause of this retardation he attributed to the development of a specific antioxidase, which inhibits the normal activity of the oxidase of the root tip.

Reducing properties have been found in animal organs, in microorganisms, and in plant juices. The reducing power of the animal organism was shown clearly by Ehrlich,[c] who used alizarin blue and the more easily reduced indophenol blue, both of which on reduction give colorless bodies. On injecting these colored substances, he found that organs of the animal could reduce them to the leucobases. Ehrlich divided the organs of the body into three main groups: (1) Those highly saturated with oxygen in which indophenol blue remains; (2) those in which indophenol blue, but not alizarin blue, is reduced; (3) those of highest oxygen avidity in which alizarin white is formed.

In the first group are the gray matter of the nervous system, heart, part of the muscular system, and kidney capsule; in the second group most of the muscular system, glands, and connective tissue; in the third group, the lungs, liver, fat, Harder's gland, and mucosa of the stomach.[d]

Since Ehrlich's experiments the reducing power of animals, plants, and microorganisms has been extensively studied. The reagents

[a] Ber. deutsch. bot. Ges., **20,** 454 (1902).

[b] Jahr. wiss. Bot., **43,** 145, 361 (1906).

[c] Das Sauerstoffbedürfniss des Organismus. Berlin, 1885.

[d] See Ehrlich, loc. cit., pp. 113–119.

used to demonstrate the reducing power are numerous. Among them are various colored bodies which form leuco-bodies, such as lacmus, methylene blue, indigo carmine, indigo blue, gentian violet, methyl violet, rosaniline, etc.; nitrates, which are reduced to nitrites; sodium selenite and sodium tellurite, which are reduced to metallic selenium and tellurium. Methylene blue and lacmus are used most. The former is converted to a leuco-base by the addition of hydrogen, the latter apparently by the abstraction of oxygen.

Reduction has been studied more extensively in the case of microorganisms than in animals and plants. Whether the reduction produced by microorganisms is due to enzymes or not has not yet been settled. Rozsahegyi,[a] Baginsky,[b] Müller,[c] and Wolff[d] believe that the reduction is caused by external metabolic products, while Cahen,[e] Spina,[f] Smith,[g] Klett,[h] Maassen,[i] and Cathcart and Hahn[j] regard it as an attribute of the bacterial cell. They all agree, however, that the reduction is associated with the development of bacteria.

In regard to reduction in higher plants, it might be mentioned that Binz and Schulz[k] found that plant protoplasm had the power of reducing arsenic acid (As_2O_5) to arsenious acid (As_2O_3), while plant protoplasm treated with boiling water lost this power to a great degree. Rey-Pailhade[l] extracted from yeast cells by means of alcohol a substance which would form hydrogen sulphide from sulphur. To this substance he gave the name philothion. He found it likewise in fresh animal tissues, in the tips of young shoots of asparagus[m] and in various grains and young seedlings.[n] Pozzi Escot[o] verified Rey-Pailhade's work and succeeded in hydrogenating in addition metallic selenium and phosphorus. Palladin[p] found a reductase in wheat sprouts and concluded that it played a part in respiration. Deleano[q] found a reductase or hydrogenase in the albumen of *Ricinus communis*, but not in the roots or the plantules. The albumen triturated with sulphur gave hydrogen sulphide. Laurent[r] showed that young seedlings reduce nitrates and that there can be extracted from plants an unorganized ferment, which has the power to convert nitrates to nitrites. Abelous and Aloy[s] found a

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[a] C. f. B. I, **2,** 418 (1887).
[b] Deutsche Med. Wochenschr, 391 (1888).
[c] C. f. B. I, **26,** 51, 801 (1899).
[d] C. f. B. I, **27,** 849 (1900).
[e] Zeit. Hyg., **2,** 386 (1887).
[f] C. f. B. I, **2,** 71 (1887).
[g] C. f. B. I, **19,** 181 (1896).
[h] Zeit. Hyg. **33,** 137 (1900).
[i] Arb. Kais. Gesundheitsamt, **18,** 475 (1902).
[j] Arch. Hyg., **44,** 295 (1902).
[k] Arch. f. exp. Path. **11,** 200 (1879).
[l] Compt. Rend. **106,** 1683 (1888).
[m] Compt. Rend. **107,** 43 (1888).
[n] Compt. Rend. **121,** 1162 (1895).
[o] Bul. Soc. Chim., de Paris, **27,** 346 (1902).
[p] Ber d. bot. Ges. **26A,** 125 (1908).
[q] C. f. B. II, **24,** 130 (1909).
[r] Ann. l'Inst. Pasteur, **4,** 722 (1890).
[s] Compt. Rend. Soc. Biol., **55,** 1080 (1903).

nitrate-reducing enzyme in potato tubers. Kastle and Elvove[a] confirmed the presence of the nitrate-reducing enzyme in the potato and showed that it was present also in the fruit of the eggplant (*Solanum melongena*). Recently Irving and Hankinson[b] have found nitrate-reducing enzymes in certain water plants.

Since little has been done upon the reducing power of seedlings growing in soil or solutions, experiments were made to determine the power of the intact and growing roots, especially of wheat, to reduce substances, with the ultimate purpose of seeing if, like the oxidative power, the reducing power would be found to play a significant part in soil fertility.

Various dyes, such as methylene blue, indigo carmine, Bismarck brown, gentian violet, etc., were first employed to see if the roots would decolorize the solution in which they were growing. The color of dilute solutions of the dyes was greatly lightened by the growing roots, especially if air was excluded. Since, however, more or less of the dyestuff was deposited on the outer surface of the root and root hairs, such solutions were not considered satisfactory for showing reduction.

Starch iodide solution.—When wheat seedlings are placed in dilute solution of blue starch iodide with seeds and roots in the solution, the blue color is soon discharged. With the roots only in the starch iodide solutions, the color is discharged slowly. The addition of hydrochloric acid to the decolorized solution brings back the blue color. Reducing agents such as sodium thiosulphate, formaldehyde, hydrogen sulphide, etc., behave similarly.

Seedlings were kept in water solution of iodine of 0.012 per cent strength of iodine for two hours with (A) seeds and roots in iodine solution; (B) roots only in iodine solution; and (C) control in which no plants had been. Solution (A) gave no color on addition of the starch, (B) gave a slight blue color, and (C) gave a deep blue.

On the addition of a few drops of concentrated hydrochloric acid all the solutions became deep blue and no difference could be seen in the depth of color. It would seem, then, that the plants hydrogenate the iodine, forming an iodide from which the acid liberates the iodine, or that the plants in some way render the iodine inactive toward the starch.

Since the chemistry of the starch-iodine combination is not sufficiently known and since the intensity of color produced by the reaction between starch paste and iodine is by no means quantitative, the effect the seedlings had on this reaction was taken merely as a sign of the possibility of a hydrogenase or a reductase in the seedlings, seed, or root.

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[a] Am. Chem. Jour., **31**, 609 (1904). [b] Biochem. Jour., **3**, 87 (1908).

Sulphur.—With the seedlings intact the roots and seeds in one case and the roots alone in another were put in contact with well-washed precipitated sulphur, neutral, made slightly acid with hydrochloric acid, or slightly alkaline with sodium hydrate. In no case was hydrogen sulphide detected by means of lead acetate paper. Alcoholic extracts of the crushed seedlings after the method of Rey-Pailhade[a] and Pozzi-Escot[a] were likewise found to have no hydrogenating action on precipitated sulphur.

Heffter[b] found that compounds containing the sulphydryl or SH group, such as thiophenol, benzylmercaptan, ethylmercaptan, thioglycolic acid, and cystein, reduce sulphur, and came to the conclusion that the reducing power of cells is due to the labile hydrogen of sulphydryl compounds which give up their hydrogen and become disulphides. This sulphydryl group is present in certain albumins and is shown by a purple red coloration with sodium nitroprusside and sodium or ammonium hydrate. The crushed wheat roots or seedlings do not give this reaction. According to Heffter nonreducing albumins can be changed to reducing albumins by treatment with a 10 per cent sodium sulphite solution for twelve to twenty-four hours and will then give the nitroprusside reaction.

Wheat seedlings of different stages of development, from three to ten days old, were rapidly crushed, covered with a 10 per cent solution of sodium sulphite, and kept at 40° C., with repeated shaking. The solutions with suspended matter were tested after two, six, and twenty-four hours and at no time gave the nitroprusside reaction for the sulphydryl group, nor did they reduce sulphur. In twenty-four hours the seedling pulp was tested for the sulphydryl group, with negative results, and the pulp formed no hydrogen sulphide from freshly precipitated sulphur.

Nitrates.—To test the power of wheat seedlings to reduce nitrates to nitrites the wheat grains were treated with a 0.1 per cent solution of mercuric chloride for thirty minutes and the seeds, well washed with sterile water, were placed in sterile tubes containing little water. In the tubes tightly closed with cotton the wheat germinated and grew well. Ten cubic centimeters of a 1 per cent solution of potassium nitrate was added to each tube and after twenty-four hours the solution was tested for nitrites by means of Griess reagents,[c] sulphanilic acid, and naphthylamine acetate. The ungerminated seeds, whether sterilized or not, did not give the nitrite test under these conditions. The solutions containing the seedlings, on the other hand, gave good tests for nitrites. The ability of sterile seeds germinated in tubes to form nitrites from nitrates was tested several times, both in air and in vacuum. Sometimes a fair

[a] Loc. cit.

[b] Med. naturwiss. Arch., **I,** 81 (1907–8).

[c] Bul. 31, Bureau of Soils, U. S. Dept. Agr., p. 41.

amount of nitrite was formed, in one case as high as 6 parts per million; sometimes the amount was very little. It should be borne in mind, however, that nitrites are absorbed by the plants. The formation of nitrites was greater in vacuum. It would seem that a reducing power appears in the early stages of the wheat seedling, but is not in the seed at rest. At best, however, the formation of nitrites is small and it is not certain that microorganisms were entirely excluded in the experiment. It may be said, however, that enzymes capable of reducing nitrates have been reported in plant tissue by Laurent,[a] Abelous and Aloy,[a] Kastle and Elvove,[a] and Irving and Hankinson,[a] and further, that Laurent claims that young seedlings of maize, white lupine, and peas have a reducing action on nitrates, while Irving and Hankinson have found that a number of plants reduce nitrates more or less.

Sodium selenite.—Sodium selenite was next employed as a test solution. Solutions of sodium selenite of 0.25 per cent were first used. The solutions reacted alkaline to litmus and phenolphthalein and were toxic to the young seedling. When neutralized by hydrochloric acid, however, the toxicity of the selenite was greatly lessened. When seedlings were grown in the sodium selenite solution made neutral or slightly acid to phenolphthalein and of a strength of 0.125 to 0.25 per cent, the parenchyme cells of the end of the root next to the root cap were colored an intense pink in a few hours, varying with the seedlings, by the deposit of selenium. This deposit is more marked in slightly acid solutions. The points of emergence of the secondary roots were likewise colored. Later the whole root became colored.

Sodium tellurite.—When sodium tellurite is used as a test for reduction, the roots are colored a blue-black by the deposit of metallic tellurium, otherwise its behavior is like that of sodium selenite.

These experiments show conclusively that the intact roots possess a reducing power. This reducing power is stronger in the young seedling, being much stronger in the seedlings 4 days old than in the seedlings 12 days old. As judged by the quickness with which the deposit of selenium is made on the roots and the extent and intensity of the deposit, the reducing power increases from time of germination to the sixth or eighth day and then decreases. It is still present in seedlings 13 days old, the oldest seedlings examined. The oxidizing power of the wheat seedlings, on the other hand, as judged by the oxidation of aloin, is less in the young seedling and increases with age, being considerably greater in the 12-day seedlings than in the 6-day seedlings and still greater than in the 4-day seedlings.

Dying tissue tends to have a reducing action. The reducing action of the wheat roots on sodium selenite, however, is not a death phe-

[a] Loc. cit.

nomenon, since (1) roots killed by being dipped in boiling water have no reducing action on the selenite; (2) the roots in toxic, nonneutralized sodium selenite do not reduce; (3) intact roots boiled in solution of sodium selenite have no deposit of selenium upon them; (4) the root ends being cut off and the injured root placed in the selenite solution, the deposit of selenium is not on the cut end but at the point of emergence of the secondary roots.

The reducing power of the wheat roots on sodium selenite is checked by acids and alkalies and by toxic organic matter. It is stimulated by faintly acid reaction and by light. The observable action of salts on the reducing action is variable on account of lack of precision of method, though as a rule sodium nitrate solution (equivalent to 50 parts NH_3 per million) gives the quickest and heaviest deposit at the root tip, while potassium salts give the heaviest deposit on the rest of the root. Potassium salts containing 50 parts K_2O per million appeared to retard reduction slightly at the root tip. Potassium iodide containing 14.5 parts K_2O per million, however, stimulated reduction.

Incidentally, it is of interest here to state that potassium greatly retards the oxidative power, as indicated by aloin, and that a slight acid reaction stimulates reduction while inhibiting oxidation, thus seeming to show that the reducing and oxidizing powers are not necessarily concomitant.

Whether or not the reducing power of the wheat roots on sodium selenite is due to enzyme activity, it is hard to say positively. In regard to the enzyme nature of the reducing agent in animal cells, Heffter [a] concluded that there were two groups of reduction processes. One group was hardly influenced by hydrocyanic acid or by heating. This group embraced the formation of hydrogen sulphide from sulphur, the reduction of arsenic, tellurium, and selenium compounds, the reduction of cacodylic acid and of different dyestuffs. These reductions, he believes, are caused by the labile hydrogen of the sulphydryl group of certain albuminous bodies. The other group of reduction processes, such as the reduction of nitrates and the change of nitrobenzol to amino combinations, was restrained by hydrocyanic acid and completely inhibited by heat. This group of reactions he thought at first might be enzymotic, but later he [b] came to the conclusion that they likewise were nonenzymotic.

Whether enzymotic or not, the reducing action on sodium selenite is most marked intracellularly in the parenchyme cells of the root tip. The primary localization of the deposited selenium in the root tip speaks neither for nor against the enzyme nature of the reducing power.

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[a] Med. naturwiss. Arch., **1**, 81 (1907).

[b] Arch. f. exp. Path., Supplement, 1908, 253.

Reduction of sodium selenite may be brought about by purely chemical means. Thus Grüss[a] found that lactic acid could precipitate selenium from sodium selenite. Lactic acid we have found to have no reducing action on sodium selenite of a strength of 0.25 per cent, but has some reducing action on a 4 per cent solution, especially on warming. Citric, tartaric, malic, and oxalic acids do not reduce sodium selenite in the cold, but do reduce strong solutions on boiling with a slight excess of the acid. Hydrochloric acid has no reducing action on the selenite solution. Citric, tartaric, malic, and oxalic acids have a reducing action likewise on ferric salts, converting them to the ferrous form. Unsaturated fatty acids, such as oleic and elaidic, also have the power to reduce slightly acid solutions of sodium selenite and tellurite to metallic selenium and tellurium on warming the mixtures on the water bath. Dextrose likewise reduces the selenite and tellurite on warming. A strong solution of sodium selenite mixed with a solution of invert sugars made by warming cane sugar with dilute hydrochloric acid was completely reduced in the slightly acid mixture on standing forty-eight to seventy-two hours at the room temperature of about 30° C.

Jones[b] injected selenate into the blood of animals. The selenate was reduced to selenite, a small part of which was excreted in the urine. The remainder was carried to the spleen and liver, where it was reduced by dextrose to selenium. When the dextrose was exhausted fat was called upon. Jones suggests that dextrose is possibly the means by which all reduction processes in the body are brought about. He found that arabinose, glucose, and sugars yielding glucose would reduce sodium selenite on heating, while levulose could reduce it at 30° C. on long standing.

As regards the reduction of sodium selenite and tellurite by wheat roots, it seems probable, since no reducing enzyme could be extracted from the crushed plant, although the juice does reduce the selenite and tellurite on heating, that the reduction is due to the metabolic activities of the roots, to some unstable nonenzymotic bodies comparable to the organic hydroxyacids which have a slight reducing power, or to complex unsaturated compounds comparable in properties to dextrose and levulose or the unsaturated fatty acids.

In regard to the significance of the reducing power, it may be said that reduction in organisms runs in general concurrently with oxidation and is probably just as important an index of the life activity. A better medium than sodium selenite is required, however, for a detailed study of this reducing power. Such a medium would be a chromogen which changed color under the reducing action and thus

[a] Ber. deutsch. bot. Ges., **26a,** 627 (1908).
[b] Biochem. Jour., **4,** 405 (1909).

would present a possibility of estimating the change colorimetrically and quantitatively. The fact stands, however, that the growing root possesses reducing powers. The next step is to see what relation reduction in plants bears to oxidation.

CONCURRENT OXIDATION AND REDUCTION BY ROOTS.

Ehrlich,[a] whose results have been verified by others, has shown that oxidation and reduction processes may exist side by side in the animal organism. According to him, the functioning protoplasm can oxidize or reduce by means of its oxygen saturated or unsaturated compounds, respectively. Since the difference in metabolism between plants and animals is one of degree only, it is to be expected that the plant cell likewise should exhibit both oxidizing and reducing powers, as has been found to be the case. Oxidation has been more extensively studied on account of its more readily recognized significance in life functions. Reduction, however, as said before, is probably just as important as oxidation in the economy of the living cell and is probably just as good an index of life activity. It is most likely that in the living cell oxidation and reduction alternate in quick succession, as Drechsel[b] has shown to be the case in certain oxidations outside the living body. It is probable that in this continual oxidation and reduction, protoplasm suffers no change in its essential properties and thus preserves its identity.

Two classes of substances have been found useful in showing the oxidizing power of roots in solution culture. The first class comprises certain soluble chromogens which yield upon oxidation insoluble compounds mainly deposited upon the surface of the roots. The second class of chromogens consists of certain substances which give soluble coloring matter as the result of the oxidizing action of the roots, shown by the change from a colorless to a colored compound or by a change from one color to another which is distinctly different. Compounds belonging to the first class are alphanaphthylamine, benzidine, vanillin, vanillic acid, and esculin. The second class of chromogens is in many respects more useful for oxidation studies because the intensity of the color, and hence the amount of oxidation, can be quantitatively expressed. Among these substances are phenolphthalin, aloin, and leucorosolic acid.

When organic substances like alphanaphthylamine and benzidine in solutions of 10 parts per million and 5 parts per million, respectively, are used, the colors due to oxidation are shown on the root. The most marked oxidation is shown by a narrow but very distinct

[a] Das Sauerstoffbedürfniss des Organismus, 1885.

[b] Jour. prakt. Chem., (NF) **22**, 476 (1880); **29**, 229 (1884); **38**, 65 (1888).

band of color just back of the root cap. Then comes a practically colorless zone and then a broad colored zone, the color becoming less intense toward the upper part of the root. When a 0.04 per cent aloin solution is used, a diffusion zone of color is at first formed close to the root, but in the course of a few hours the solution, as a whole, becomes a cherry red, which varies in depth according to the amount of oxidation.

Reduction by roots has been studied but little. Reduction by the tissues of animals and by microorganisms, on the other hand, has been extensively studied. The reagents used to indicate the reducing power of tissue are chromogens, which on reduction by abstraction of oxygen or by the addition of hydrogen, yield a leuco base, such as alizarin blue, indophenol blue, lacmus, methylene blue, indigo carmine, rosanilin, gentian violet; nitrates which are reduced to nitrites; sodium selenite and tellurite, which are reduced to metallic selenium and tellurium; sulphur which is converted to hydrogen sulphide.

To show reduction we have tried various reagents and have found sodium selenite to be the best medium at our command, as already mentioned. In solution of sodium selenite made N/500 acid by hydrochloric acid, the deposit of selenium at the tip of the root was very marked. In alkaline solution the reduction was very little. In short, reduction is more marked in slightly acid solutions. Oxidation, on the other hand, is stronger in slightly alkaline solutions.

As judged by the quickness with which the deposit of selenium is made on the root and the extent and intensity of the deposit, the reducing power increases from the time of germination to the sixth or eighth day and then decreases. It is still present in seedlings 13 days old, the oldest seedling examined. The oxidative power of the wheat seedling, on the other hand, as judged by the oxidation of aloin, is less in the young seedling and increases with age.

The relative oxidation was determined by placing the seedlings in culture bottles, 10 seedlings to a bottle, with the roots in the aloin solution made by dissolving 400 milligrams in 1 liter of pure distilled water. In twenty hours the depth of color produced by the red oxidation product was measured in the colorimeter. Taking the reading for the 12-day seedling as 100, the relative oxidation of the 8-day seedling was 81; of the 5-day seedling 48.

Oxidation of aloin by roots is greater in darkness than in light, the relation being in three separate tests with 4, 6, and 9 day seedlings 100 in the darkness to 75, 70, and 71, respectively, in light.

Nitrates and phosphates increase the oxidizing power of the wheat roots, while potassium salts, especially potassium iodide, decrease it.

TABLE I.—*Effect of salts on oxidation of aloin by roots.*

Solutions.		Relative oxidation.
Control in pure distilled water		100
Sodium nitrate	50 p. p. m. NH_3	135
Potassium sulphate	50 p. p. m. K_2O	42
Acid calcium phosphate	50 p. p. m. P_2O_5	117
Sodium nitrate Potassium sulphate Acid calcium phosphate	50 p. p. m. NH_3, K_2O, P_2O_5	100
Potassium chloride	50 p. p. m. KCl	38
Potassium iodide	50 p. p. m. KI	20
Iodine	50 p. p. m. I	16

In the experiment given in Table I the seedlings were started germinating January 4 and were put into the nutrient solutions January 10. On January 14 the aloin was placed in the solution and on January 15 the oxidation readings were made.

The accelerating effect on oxidation of a sodium nitrate solution containing the equivalent of 50 parts NH_3 per million and the retarding effect of potassium salts equivalent to 50 parts K_2O per million is shown likewise in the case of plants growing in darkness. In this case the plants 6 days old were put into the salt solutions containing 0.04 per cent of aloin and the experiment continued for twenty-four hours, when the depth of the red oxidation product was compared in the colorimeter with the oxidation products of plants growing in carbon-treated water plus aloin as a control. (Table II.)

TABLE II.—*Relative oxidation of aloin by roots in darkness and in light.*

Solutions.	In darkness.	In light.
Control	100	70
Sodium nitrate	150	105
Potassium sulphate	70	47
Potassium chloride	91	60
Potassium iodide	66	37

Since Bielecki [a] has shown that nitrates of potassium, ammonium, and calcium added to a solution of peroxidase leads to its passing through a dialyzer in amounts proportionate to the amount of salt added, it may be that the nitrates increase the oxidizing activity of the wheat roots by allowing more of the oxidizing substance to pass into the medium. With alcoholic guaiac as an indicator, we have found a strong peroxidase reaction in the water in which the seedling roots had grown for twenty-four hours, and a stronger reaction in sodium nitrate solution in which plants have grown the same length of time. This peroxidase reaction does not occur if the culture water is boiled.

[a] Biochem. Zeit., **21**, 103 (1909).

The observable action of salts on the reducing action is variable on account of lack of precision of method, as already mentioned. The potassium salts which retard oxidation have little effect on reduction. Thus a potassium iodide solution containing 14.5 parts K_2O per million practically inhibited the oxidation of aloin by the roots of five-day-old wheat seedlings, while allowing good reduction of sodium selenite. It would seem then that reduction and oxidation may be independent of each other.

Attempts were made to show simultaneous oxidation and reduction by placing the seedlings with the roots in mixtures of sodium selenite and aloin, benzidine, and alphanaphthylamine, respectively. In mixtures of sodium selenite and alphanaphthylamine the two deposits of color on the roots were so nearly alike that it was difficult to tell whether selenium had been deposited or not in connection with oxynaphthylamine. In solution of benzidine (5 parts per million) the roots as a whole had a blue-black appearance and a heavy ring appeared just back of the root cap. In the same strength of benzidine plus 0.25 per cent sodium selenite, N/1,000 acid to phenolphthalein, the black oxidation ring at the root tip still appeared, but the roots, as a whole, were pink from the deposited selenium. In 0.04 per cent aloin and 0.15 per cent sodium selenite made N/500 acid some oxidation of the aloin solution occurred and a good reduction of selenite. In aloin and sodium selenite N/1,000 acid there was strong oxidation and slight reduction.

The experiments show that it is possible to have oxidation and reduction by the same roots in the same solution. A slightly acid reaction is favorable to reduction, a slightly alkaline reaction is favorable to oxidation. Between these acid and alkaline limits both processes are demonstrable as occurring side by side.

It has long been supposed that oxidation by the plant and plant extracts is due to the presence of oxidizing enzymes such as the oxidase, peroxidase, and the related catalase. A good review of the literature on cellular oxidation with consideration of the relationship between the enzymes may be found in the works of Bach[a] and Kastle.[a]

According to Bach the oxidase is a mixture of peroxidase and a peroxide forming substance. The enzymes taking part in cellular oxidation are, according to him, oxygenase, peroxidase, and catalase. He explains their relationship as follows: (1) In order to have at its disposition a continued source of active oxygen, the living cell produces oxygenases or bodies capable of fixing atmospheric oxygen with the intermediary formation of peroxides; (2) the peroxides thus formed are activated by the peroxidase in the way that hydrogen

[a] Loc. cit.

peroxide is activated by ferrous sulphate. The system peroxidase-peroxide effects the oxidation of substances serving as aliments to the cell, substances which are oxidized with difficulty; (3) in cases which are particularly favorable to the formation of peroxide, excess of which might be injurious to the cell, the cell generates a third ferment, catalase, which decomposes the peroxide with the liberation of molecular oxygen.

According to Moore and Whitley[a] there is little evidence of the existence of Bach's oxygenase. These workers believe that all juices showing oxidizing properties possess one type of ferment which, since it acts only in the presence of either naturally occurring or artificial peroxide, may be styled a peroxidase. According to them there is no proof of the existence of any other type of enzyme engaged in oxidizing processes.

In regard to cellular oxidation it must be said that nothing absolutely certain is known regarding the composition or nature of the oxidizing substances in plants and the chemistry of the oxidizing bodies is uncertain. Apart from their destruction by heat and by poisons little is known concerning their enzymotic nature. They do not seem to act as oxygen carriers in the sense of being able to transfer large amounts of oxygen from the air to the oxidizable substance, since the amount of oxidation is increased only slightly by an increase in the amount of oxygen present. Thus the relative oxidation by the roots of plants growing four hours in an atmosphere of pure oxygen and of air, respectively, was 100 and 80. The relative oxidation by plants in air and in a partial vacuum of a pressure of 23 millimeters of mercury for four hours was 100 and 66; growing nineteen hours in a partial vaccum, 100 and 74, respectively. In an atmosphere of carbon dioxide oxidation was begun early, but this then stopped, and in sixteen hours was a mere trace compared to the oxidation by plants growing in aloin solutions in air. Oxygen is necessary for plant oxidation, but a great increase in the amount of oxygen does not greatly increase the oxidation by plants.

Many, if not all, of the oxidations produced by the so-called enzymes can be brought about by nonenzymotic bodies, both organic and inorganic, as first shown by Schönbein.[b] Organic bodies like benzaldehyde, benzoyl peroxide, and succinyl peroxide, pyrogallic acid, and quinone behave like an oxidase, as do peroxides and other salts of inorganic bases such as iron, lead, manganese, etc.

Spitzer[c] attributes the oxidizing power of animal tissues to the nucleoproteid they contain, the oxidizing power of the nucleoproteids being due to their combined iron. In the absence of sufficient oxy-

[a] Biochem. Jour., **4**, 136 (1909). [b] Jour. prakt. Chem., **102**, 145 (1867).
[c] Pflügers Arch., **67**, 615 (1897).

gen the same nucleoproteid acts as a reducing agent. Bertrand[a] showed that the oxidizing power of laccase from the lac tree is associated with the presence of manganese, and that laccase of alfalfa, which is poor in manganese, is inactive toward hydroquinone.[b] The mixture of a trace of manganese salt and alfalfa laccase had strong oxygen-carrying power where either constituent alone had very little. Other metals, such as iron, aluminum, cerium, zinc, copper, calcium, magnesium, and potassium, did not increase the oxidizing power of laccase. Bertrand showed that manganese salts, which are easily hydrolyzable, are most efficient oxygen carriers. He considered the active oxidase as special combinations of manganese and an acid radical, the latter probably of a proteid nature and partaking of the properties of a ferment. The manganese would be the really active element of the oxidase, while the acid radical would give the ferment the other properties such as solubility and sensitiveness to heat.

According to Rey-Pailhade[c] the reducing ferment which he has described under the name "philothion" possesses the properties of the acid albuminoid radical of the oxidases.

In connection with Bertrand's conception of the action of manganese and our study of the oxidative action of wheat roots, it is interesting to note that manganese has been found by Maumené[d] in many plants, among them wheat, as a salt of an organic acid. Pichard[e] and Gössl[f] found manganese widespread in plants and animals. On the other hand, Vadam[g] found no manganese in the oxidase of hellebore, but did find iron; Sarthou[h] found only calcium, iron, and sodium in the oxidase of *Schinus molle*, while De Stoecklin[i] could not find manganese in the ash of the oxidase from horseradish. Bach[j] believes he has obtained from molds an active oxidase which is entirely free from iron or manganese. Van der Haar,[k] however, doubts the validity of Bach's conclusion as regards the absence of manganese. Euler and Bolin[l] found that laccase from alfalfa previously studied by Bertrand consisted of the neutral salts, mainly calcium, of certain polybasic organic acids, among which glycollic, mesoxalic, citric, malic, and probably glyoxylic acids have been determined.

Numerous inorganic compounds will change tincture of guaiac blue. Among these Schönbein[m] mentions the oxides of the noble

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[a] Bul. Soc. Chim., **11,** 717 (1894); **13,** 361 (1895); **17,** 619 (1897).
[b] Bul. Soc. Chim., **17,** 621 (1897).
[c] Bul. Soc. Chim., **17,** 756 (1897).
[d] Compt. Rend., **98,** 1416 (1884).
[e] Compt. Rend., **126,** 1882 (1898).
[f] Bot. Centr., Beiheft., **18,** 119 (1905).
[g] Jour. Pharm., **9,** 515 (1899).
[h] Jour. Pharm., **11,** 482, 583 (1900); **12,** 104 (1900).
[i] Bot. Centr., **107,** 6 (1908).
[j] Ber. deutsch. Chem. Ges., **43,** 364 (1910).
[k] Ber. deutsch. Chem. Ges., **43,** 1321 (1910).
[l] Zeit. phys. Chem., **69,** 187 (1909).
[m] Jour. prakt. Chem., **102,** 155, (1867).

metals, the so-called superoxides, saltpeter, chromic acid, permanganic acid, etc., lead peroxide, bromine, chlorine, etc. Alsberg[a] has shown that many salts have the power to change guaiac to a blue oxidation product either directly or with the addition of hydrogen peroxide. Martinand[b] finds that oxides of alkalies and alkaline earths which can form peroxides and percarbonates fix oxygen of the air in an active form and form bodies which give reactions like organic oxidases. Salts of the oxides of metals possessing several degrees of oxidation give the oxidase reaction in their maximum oxidation. The oxidation was retarded by certain salts and especially by traces of sulphuric acid. Wolff[c] found that colloidal ferrocyanide of iron exerted in certain oxidations a catalytic effect similar to that of natural peroxidases. According to Wolff and De Stoecklin[d] ferrocyanides and sulphocyanates of iron can produce all the oxidation which can be effected by natural peroxidases, each producing specific oxidations not characteristic of the other. De Stoecklin[e] later found that tannate of iron in the presence of hydrogen peroxide can bring about oxidation of such compounds as generally resist the action of any of the natural peroxidases now known. Among the substances were phenols, cresol, thymol, anisol, carvacrol, guaicol, pyrogallol, eugenol, isoeugenol, and tyrosine.

In work with aloin we found that a solution of ferric chloride containing 3 parts Fe per million gave good oxidation of aloin. Ferrous chloride even in solutions containing 25 parts Fe per million did not oxidize aloin without the addition of hydrogen peroxide. With the addition of hydrogen peroxide 1 part Fe as $FeSO_4$ per million strongly oxidized the aloin. Other salts which have a direct oxidizing action on aloin are manganese dioxide, calcium oxide and carbonate, and magnesium oxide. Aluminum sulphate and chloride in 5 parts per million of solution oxidized the aloin with the addition of hydrogen peroxide. Sodium and potassium hydrate oxidized the aloin slowly. Copper sulphate plus sodium chloride oxidized aloin solutions immediately. On the organic side benzaldehyde, quinone, piperidine, atropine, etc., oxidize aloin solutions readily.

From what has been said it can be seen that there is a strong similarity between the oxidizing action of wheat roots and catalyzers like inorganic salts and benzaldehyde and quinone. Probably the similarity is one of analogy only, the methods of oxidation being but different modes of oxygen transference to easily oxidizable bodies.

Kastle and Loevenhart[f] concluded that the so-called oxidizing ferment of the potato is not a true enzyme, but is an organic peroxide.

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[a] Arch. exp. Path., Supplement 1908, 39.
[b] Compt. Rend., **148,** 182 (1909).
[c] Compt. Rend., **146,** 1217 (1908).
[d] Compt. Rend., **146,** 1415 (1908).
[e] Compt. Rend., **147,** 1489 (1908).
[f] Am. Chem. Jour., **26,** 539 (1901).

They believe that the oxidation phenomena occurring in plants and probably also in animals can be satisfactorily explained upon the supposition that the readily autoxidizable substances which they contain are oxidized to the peroxide condition by molecular oxygen and that the peroxides thus formed in turn give up part of their oxygen to other less oxidizable substances present in the cell. In other words, the process of rendering oxygen active by the living cell is probably brought about in essentially the same way that this is accomplished by phosphorus, benzaldehyde, and oxygen carriers generally.

In regard to reduction by the living cell, likewise, uncertainty exists as to whether it is brought about by a true enzyme or by nonenzymotic bodies. Heffter [a] has given this question much attention and has come to the conclusion that the reducing activities of organisms are not due to enzymes. As in the case of oxidation, reduction processes comparable to those of the living root may be brought about by purely chemical means, as has been previously shown.

As regards oxidation and reduction, it may be said that oxidative bodies may be extracted from the plant roots by suitable means. No body which would reduce sodium selenite in the cold could be extracted, however, although the water extract of the seedlings reduced the selenite on heating. Thus it seems that the reduction of the selenite by the wheat roots is due to the metabolic activities of the roots per se, and is probably due to the presence of some unsaturated compounds, comparable in properties to unsaturated fatty acids, dextrose, levulose, etc., which are readily oxidized in contact with air, or to substances like organic hydroxyacids and their salts, which possess a slight reducing action.

In regard to the significance of the oxidation and reduction in roots, we may say that whatever increases the development of the plant increases oxidation by the roots and contrariwise whatever decreases oxidation by the roots, decreases the growth of the plant. In a word, oxidation is closely connected with the metabolic activities of the roots. It has been shown [b] that in extracts from productive soils oxidation by roots was strong and in extracts of certain poor soils the oxidation was weak, so that from a soil-fertility standpoint oxidation by roots has considerable interest. Oxidation is due undoubtedly to bodies capable of fixing atmospheric oxygen in active form, perhaps as peroxides, which secondarily oxidize bodies by the transfer of active oxygen to them. Reduction is probably brought about by nonenzymotic compounds analogous to the organic hydroxyacids and their salts, which have a reducing action, easily demonstrable by their changing of ferric iron to ferrous iron, or to compounds unsatu-

[a] Med. naturw. Arch., I, 81 (1907–8); Arch. exp. Path., Supplement, 1908, 253.
[b] Bul. 56, Bureau of Soils, U. S. Dept. Agr.

rated in respect to oxygen, which by their avidity for oxygen reduce the oxygen-containing compounds they come in contact with. Oxidation processes are weak in the young seedling, but increase in strength as the seedling grows. Reduction processes, on the other hand, are predominant in the early stages of the seedling growth, but are less manifest as the seedling develops and oxidation becomes predominant. In certain stages the two processes occur together and can be made manifest either independently or concurrently. Reduction seems to be mostly intracellular. Oxidation, on the other hand, is manifested strongly extracellularly and seems to be by far the more prominent property of the plant root.

Since oxidation by the roots is active in the medium in which the plant is growing, and since, further, the solution in which the plants have grown have more or less oxidizing power, it became of interest to see whether soil, the natural habitat of plants, possessed an oxidizing power per se or coincident with the growth of plants or subsequent to such growth.

OXIDATION IN SOIL.

The advantage of aerating soils has long been known and is well recognized in the practice of tillage. Liebig[a] early pointed out the importance of a thorough aeration of soils. According to him, "in a soil to which air has no access, or at most but very little, the remains of animals and vegetables do not decay, for they can only do so when freely supplied with oxygen, but they undergo putrefaction, for which air is present in sufficient quantity. The frequent renewal of air by plowing and the preparation of the soil, especially in contact with alkaline metallic oxides, the ash of burnt coal, burnt lime, or limestone, change the putrefaction of its organic constituents into a pure process of oxidation; and from the moment at which all the organic matter existing in a soil enters into a state of oxidation or decay its fertility is increased." According to Liebig,[b] Ingenhouss and De Saussure found that vegetable mold extracted oxygen from air with great rapidity and replaced it by an equal volume of carbonic acid.

While Liebig's observations are interesting and in the main true, it can hardly be said that he fully understood the nature of the oxidative processes he speaks of, since the possibility of decay by microorganisms was not considered by him.

Dehérain and Demoussy[c] studied the process of the oxidation of the organic matter in several soils and found that oxygen was always taken up and carbon dioxide set free. The oxidation was attributed

[a] Liebig's Complete Works on Chemistry, p. 44, 1852.
[b] Loc. cit., p. 112.
[c] Ann. Agron., **22**, 305 (1896).

by them to the action of microorganisms and to simple chemical action. Both kinds of oxidation increase with heat. Up to 65° C. the carbon dioxide increased. From 65° to 90° it decreased. Above 90° there was another increase in carbon dioxide. At 110° over six times as much carbon dioxide was formed as at 65° C. Some oxidation went on at 22° C. in soils which had been heated at 120° C. for one hour. Often more oxygen was consumed than could be accounted for in carbon dioxide formed.

Wollny [a] studied the rate of oxidation by determining the amount of carbon dioxide produced in a given time at a constant temperature. He concluded that the oxidation is due to the activity of microorganisms. Lime favored oxidation. Chlorides and sulphates retarded, while phosphates and nitrates favored oxidation.

More recently Russell [b] studied oxidation by estimating the oxygen absorbed by soils. He found that the same factors which influence fertility also influence the rate of oxidation and apparently to the same extent; that oxidation was greater in fertile soils than in infertile soils, in surface soil than in subsoil. With different soils of the same type, the rate of oxidation varies in the same way as the fertility, and may be used to measure it. Pasture soils, however, are excluded. Russell suggests that the oxygen absorbed measures the total action of microorganisms, which, by producing enzymes, etc., hasten decomposition.

As shown by Russell, the assumption that the evolution of carbon dioxide is proportional to the amount of oxygen absorbed is not proved. Many soils, as is well known, have a great power of absorbing carbon dioxide. Russell's method of measuring the amount of oxygen absorbed is a very good one, but requires a somewhat complex apparatus, takes considerable time to show differences in soils, and marks respiration of microorganisms as much as oxidative changes in the soil constituents. A simpler test of oxidative processes in soils we have found the changes produced in easily oxidizable substances brought in contact with the soil, such as aloin, pyrogallol, hydroquinone, paraphenylenediamine, benzidine, guaiac, alphanaphthylamine, which speedily show oxidative processes and have been used extensively as a test of such processes. By means of these easily oxidizable chromogenic substances the plant roots have been shown to have a strong oxidative power. This power was found to be affected by agencies which affected soil fertility, such as fertilizer salts, toxic substances, etc., and was shown to have a considerable agricultural interest in that it could affect alterations in the soil constituents and thus influence soil fertility, especially in connection with fertilizers and a system of crop rotation.

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[a] Die Zersetzung der organ. Stoffe. und die Humusbildungen. Heidelberg, 1897.
[b] Jour. Agr. Sci., **I**, 261 (1905-6).

Microorganisms, which abound in soil, likewise have been found capable of bringing about powerful oxidation of substances in the medium in which they grow. In passing it may be said that absorption of oxygen by a soil and the oxidation of readily oxidizable substances, such as the above chromogens, by the soil are not necessarily the same phenomenon. The two methods might be used together with advantage in a study of different phases of soil oxidation.

The oxidizing principles, be they enzymes or other bodies, were found in water in which the roots of growing seedlings had stood for twenty-four hours. So we should expect that by the disintegration of microorganisms and plant roots the material which brings about oxidation should be left in the soil. In addition the soil per se should have a priori an oxidizing power due to inorganic oxygen carriers like salts of iron and manganese, and probably to organic matter in an unstable, highly oxygenated state analogous to quinone, benzaldehyde, terpene bodies, or organic peroxides, which might readily give up oxygen in an active state or activate the oxygen of the air.

To test the power of the soil to oxidize, 5 grams of soil were shaken with 10 c. c. of an alcoholic solution of gum guaiac and the soil was allowed to settle. The guaiac when oxidized gave a blue product due, as shown by Doebner and Lücker,[a] to the conversion of guaiaconic acid to guaiacum blue. When thus shaken with alcoholic guaiac, the following soils immediately gave a blue color in the supernatant liquid and in the body of the soil: (1) Hagerstown loam unlimed; (2) Hagerstown loam limed; (3) limed and manured; (4) Hagerstown loam plus complete fertilizer; (5) manured;[b] (6) Hagerstown loam in permanent sod; (7) Clarksville loam; (8) Dekalb silt loam. The blue coloration was deepest in (3) and (5). This blue oxidation product gradually faded, but was more persistent in the limed soils. When faded, the addition of 0.5 c. c. of a 2 per cent hydrogen peroxide solution to the mixture brought back the intense blue color.

The soils just mentioned had all been air-dried in the laboratory—the Hagerstown loam in sod, Dekalb silt loam, and Clarksville loam for several months; the others for two weeks.[c]

The following soils did not oxidize the guaiac: Samples of Takoma lawn soil, Marshall clay loam, Norfolk fine sandy loam, Leonardtown loam, Sassafras silt loam, and Dutchess silt loam. These were air-dried samples.

Two grams of the same soils were shaken in a similar manner with 10 c. c. of a 0.125 per cent solution of aloin in 95 per cent alcohol.

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[a] Arch. der Pharm. **234,** 590 (1896).

[b] These five soils had received this treatment for many years, the last treatment being in the spring of 1909, and had been under a rotation of corn, oats, wheat, and grass at the Pennsylvania Experiment Station. The last crop was corn.

[c] Several soils air-dried in the laboratory for five years still strongly oxidize guaiac and aloin.

Only Clarksville loam and Hagerstown loam manured gave the red oxidation product. With alcoholic aloin and 0.5 c. c. of a 2 per cent hydrogen peroxide, the red oxidation product was given by Clarksville loam and samples of the Hagerstown loam, especially by the manured soil.

When 20 grams of soil were shaken with 50 c. c. of a 0.125 per cent water solution of aloin, samples of Orangeburg loam, Clarksville loam, Hagerstown loam, Hagerstown loam manured, Hagerstown loam with complete fertilizer, and Dekalb silt loam showed strong oxidation immediately. The limed soils gave a slight oxidation with a yellowish shade. The other soils gave no oxidation. To flocculate the soils and to extract the oxidized aloin, each soil and aloin mixture was shaken with 100 c. c. of 95 per cent alcohol. After the soil had settled, the supernatant liquid was poured off, centrifuged, and the depth of red color determined by means of a colorimeter. With the Hagerstown loam samples, the untreated considered as 100, the relative readings were: Check plot, 100; complete fertilizer, 107; lime and manure (6 tons of stable manure to the acre), 104; manure (10 tons of stable manure to the acre), 136. The limed Hagerstown loam produced but little red in the aloin solution, so that it could not be compared with the other soils.

Later, Arlington clay loam and Sassafras silt loam, which had been kept in a state of optimum moisture for several weeks, developed the power to oxidize guaiac and aloin. Other reagents were used, but of the reagents used as tests for oxidation none proved so suitable as a water solution of aloin. The results with the various reagents are given in Table III.

TABLE III.—*Oxidation in soils.*

Soils.[a]	Reagents.								
	Guaiac.	Guaiac+H_2O_2.	Aloin.	Aloin+H_2O_2.	Hydroquinone.	Hydroquinone +H_2O_2.	Pyrogallol.	Pyrogallol +H_2O_2.	Paraphenylenediamine+H_2O_2.
1. Clarksville loam	+	++	+	+	+sl	++	+	++	+
2. Lawn soil (Takoma)	−	−	−	−	−	−	−	?	?
3. Marshall clay loam	−	−	−	−	−	−	−	?	−
4. Volusia silt loam	−	−	−	−	−	−	−	?	?
5. Clay loam (Arlington)	+sl	+sl	+sl	+sl	−	−	+	+	?
6. Hagerstown loam in sod	+	++	+	+	+sl	++	+	++	+
7. Hagerstown loam	+	++	+	+	−	+	+	++	+
8. Hagerstown loam+lime	+	++	+sl	+	−	++	+sl	++	+
9. Hagerstown loam+lime+manure	+	++	+sl	+	−	++	+sl	++	+
10. Hagerstown loam+complete fertilizer	+	++	+	+	−	+	+	++	+
11. Hagerstown loam+manure	+	++	+	+	−	?	+	++	+
12. Norfolk fine sandy loam	−	−	−	−	−	−	−	−	−
13. Cecil fine sandy loam	−	−	−	−	−	+	+	−	?
14. Leonardtown loam	−	−	−	−	−	−	−	−	−
15. Dekalb silt loam	+	+	+	+	−	+sl	+	+	+
16. Dutchess silt loam	−	−	−	−	−	−	−	−	−
17. Sassafras silt loam	+sl	+sl	+sl	+sl	−	−	−	−	−
18. Orangeburg loam	+	+	+	+	−	+	+	+	+

[a] Only surface soils are reported. Subsoils as a rule have no oxidative power.

In the experiments given in Table III, 10 grams of soil were shaken in test tubes with 25 c. c. of 2 per cent alcoholic guaiac, 0.125 per cent water aloin, 0.5 per cent pyrogallol and hydroquinone. When hydrogen peroxide was added, it was to the extent of 0.5 c. c. of a 2 per cent solution to each tube. In case of testing with paraphenylenediamine, 25 c. c. of a 2 per cent solution of paraphenylenediamine plus 0.2 per cent hydrogen peroxide containing 1 c. c. of concentrated sulphuric acid to the liter was shaken with the soil. When oxidized the reagent develops a blue-black color. The control solution oxidized so readily, however, that it was not considered a good medium for testing oxidation and was regarded as too sensitive for differential work.

As regards the significance of the oxidation as given in the preceding pages it may be said that soils known to be productive had strong oxidative power as a rule, and that the poorer soils had little or no oxidative power. The oxidative power and productivity, however, do not necessarily agree, since we have found soils of slight oxidative power which have given the better growth as compared with soils of stronger oxidizing power. Crop production, as is well known, is dependent upon many factors, no one of which can be taken as an absolute criterion. As will be shown later, however, certain factors which favor productiveness of soils favor oxidation, so that probably there is a considerable relation between oxidation and soil fertility.

Attempts were made to extract the oxidizing principle from soils with cold and hot water, cold and hot alcohol, and ether. The cold-water extract oxidized aloin slightly in the course of a few hours, the other extracts practically not at all. The oxidation by the water extract, however, was very slight as compared to oxidation by the soil and very much slower, the aloin shaken with soil being changed to a red solution in a few minutes.

The fact that the oxidizing principles of the Hagerstown loam samples is somewhat soluble in water, but is insoluble in alcohol and is not soluble in boiling water, or is destroyed by boiling water, suggests the possibility of an oxidizing enzyme in the soil.

Woods[a] has shown that in the decay of roots, leaves, and stems of both healthy and diseased tobacco plants the oxidizing enzymes, especially the peroxidase, are liberated and remain active in soil.

Recently König[b] and others have found that soils had a catalytic power on hydrogen peroxide. They found that different soils had the ability to liberate oxygen from hydrogen peroxide. The catalytic power they attributed partly to the action of an enzyme and partly to colloidal substances, as sesquioxides of iron and manganese,

[a] C. f. B. II, **5**, 745 (1899).

[b] Landw. Vers.-Stat., **63**, 471 (1906); **66**, 401 (1907).

present in soils. May and Gile[a] attributed the hydrogen-peroxide decomposing power of soil practically altogether to an enzyme, catalase. The opinion seems to be that enzymes may exist in soils. Owing to the strong absorptive power of the soil, it would be difficult to extract them.

METHOD OF TESTING OXIDATION IN SOIL.

Since aloin dissolved in water proved to be the best medium for testing oxidation, a water solution of aloin, generally of a strength of 0.125 per cent, was employed in the further study of oxidation in soil. As a rule, 20 grams of soil were shaken four or five times in the course of an hour with 50 c. c. of the aloin solution and allowed to settle; then the mixture was treated with 50 c. c. of 95 per cent alcohol to flocculate the soil and to extract the oxidized aloin. If the oxidized aloin solution was fairly clear, the alcohol was occasionally dispensed with. The solution was then centrifuged, the supernatant liquid poured off and the depth of color in the solution compared by means of a colorimeter. The experiments from the time of adding the aloin to the soil to the reading in the colorimeter ran, as a rule, from two to three hours.

EFFECT OF VARIOUS TREATMENTS ON SOIL OXIDATION.

POISONS AND ANTISEPTICS.

To 20 grams of two soils which gave a strong oxidation of aloin—Hagerstown loam, untreated and manured—were added 30 c. c. of a 1 per cent solution of silver nitrate, mercuric chloride, oxalic acid, sodium thiosulphate, hydroxylamine hydrochloride, 30 c. c. of a saturated solution of salicylic acid, 10 c. c. of carbon bisulphide, 10 c. c. of chloroform, and 30 c. c. of a 2 per cent formalin solution. Then 50 c. c. of a 0.125 per cent water solution of aloin was shaken three or four times with each soil and the mixture allowed to stand for one hour. The oxidation was positive in the treatment with silver nitrate, mercuric chloride, carbon bisulphide, chloroform, and salicylic acid; negative in the treatment with hydroxylamine hydrochloride, oxalic acid, sodium thiosulphate, and formalin. A small amount of hydrogen-sulphide water greatly reduces oxidation.

The treated soils that showed positive oxidation of aloin were shaken with 100 c. c. of 95 per cent alcohol and the supernatant liquid decanted, centrifuged, and the depth of the red oxidation product of the aloin determined by means of the colorimeter. The comparative oxidation of the untreated soils and those treated with silver nitrate, mercuric chloride, and carbon bisulphide may be seen in Table IV.

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[a] Circular 9, Porto Rico Agr. Expt. Sta. (1909).

TABLE IV.—*Oxidation in soils treated with silver nitrate, mercuric chloride, and carbon bisulphide.*

Soil.	Treatment.			
	Normal.	Silver nitrate.	Mercuric chloride.	Carbon bisulphide.
Hagerstown loam	100	79	50	104
Hagerstown loam, manured	136	166	63	83

The experiments with substances which destroy enzymes was extended, using less carbon bisulphide and mercuric chloride and silver nitrate. The results are shown in Table V.

TABLE V.—*Effect of adding 2 c. c. carbon bisulphide, 5 c. c. silver nitrate, and 5 c. c. mercuric chloride solution on oxidation by soils.*

Soil.	Treatment.			
	Untreated.	Carbon bisulphide.	Silver nitrate.	Mercuric chloride.
Sassafras silt loam	100	100	105	97
Clarksville silt loam	100	111	95	102
Orangeburg loam	100	117	(a)	(a)
Hagerstown loam, untreated	100	114	(a)	(a)
Hagerstown loam, manured	100	91	84	90
Arlington clay loam	100	89	83	100

a Good oxidation. The oxidation color was much stronger than that of the check, but so different in shade that it was impossible to make a quantitative comparison.

These antienzymotic substances, which of themselves have no effect on aloin within the time of the experiment when put into the soil, sometimes increase, sometimes decrease the oxidative power of the soil.

Though the soil by its absorbing and combining powers renders toxic agents less effective than they would be in solution, the fact that oxidation goes on in the presence of comparatively strong solutions of mercuric chloride, silver nitrate, and carbon bisulphide would, contrary to our expectations, tend to throw some doubt on the possibility that the oxidizing action of the air-dried soils is due to enzymes. On the contrary, it would seem more probable that the oxidation of the aloin by the soils should be attributed to organic or inorganic material in the soil or to combinations of these. Nevertheless in other soils or under other conditions it may be possible that enzymes play a considerable rôle in oxidation.

DRY HEAT.

Several soils which showed a strong oxidative action on aloin solutions were heated one and one-half hours at 105° C. dry heat and, after cooling, were again tested for oxidation. The oxidation of the aloin

was reduced considerably by heating in the case of most of the soils tested. In some cases, especially in case of the Hagerstown loam, the heated soils gave such a different shade of color with so much of the original yellow of the aloin solution that their oxidative power could not be compared accurately with that of the normal soil. As read by means of the colorimeter the comparative oxidation was judged to be as follows: Clarksville loam, unheated, 100, heated, 100; Orangeburg loam, unheated, 100, heated, 111; Sassafras silt loam, unheated, 100, heated, no oxidation; Arlington clay loam, unheated, 100 (slight), heated, no oxidation; Hagerstown loam, unheated, 100, heated, 66; Hagerstown loam, manured, unheated, 100, heated, 45; Hagerstown loam plus complete fertilizer, unheated, 100, heated, 66.

The Hagerstown loam soils, Arlington clay loam, and Sassafras silt loam were heated with dry heat to 150–175° C. for two and one-half hours. They were then shaken with aloin solution, but did not oxidize the aloin at all. Clarksville silt loam treated in the same way had the oxidation reduced to a trace. Heating Orangeburg loam reduced the oxidative power of the soil considerably, the relative oxidation by this soil being, unheated 100, heated 59.

STEAM HEAT.

Heating the soils for one and two hours, respectively, in an Arnold steam sterilizer lessened oxidation in most soils, but had no retarding effect on Orangeburg loam. The relative oxidations of the unsteamed and steamed soils are given in Table VI.

TABLE VI.—*Effect of steam heat on the oxidative power of soils.*

Soil.	Unsteamed.	Steamed 1 hour.	Steamed 2 hours.
Hagerstown loam	100	65	Faint.
Hagerstown loam, manured	100	52	Faint.
Hagerstown loam, complete fertilizer	100	34	Faint.
Sassafras silt loam	100 sl.	Neg.	Neg.
Clay loam (Arlington)	100 sl.	Neg.	Neg.
Clarksville silt loam	100	54	Faint.
Orangeburg loam	100	104	105

Heating the soils in a digester for one hour at 10 atmospheres destroyed the oxidative power entirely, even in the case of Orangeburg loam.

Heat is a more effective inhibitor of the oxidative power of the soil than are the powerful antienzymotic substances such as mercuric chloride, silver nitrate, and carbon bisulphide. As compared to heat these reagents retard oxidation but little and in some soils silver nitrate and carbon bisulphide even increase oxidation. Accordingly, it is to be judged that the effect of heat on oxidation by soils is due to its effect on the soil ingredients and especially on the organic mat-

ter or some inorganic-organic complexes of the soil, since steam heating for two hours would affect strictly inorganic salts destructively but little, if at all. The organic matter of the soil, however, is known to undergo considerable modification at the temperature of 100° C. Thus Richter [a] found that heating a soil at 100° C. on three successive days converted a part of the insoluble nitrogen into a soluble form and made inorganic and organic matter more soluble. Dehérain and Demoussy [b] found that sterilization increased the ammonia content of soils and gave rise to carbon dioxide. Schulze [c] found that sterilizing soils gave rise to injurious products for a time. Later, growth became more vigorous in the sterilized soils. Kosaroff [d] found that heating changed soil both in color and smell. Solutions of the heated soils were turbid and contained more organic matter than did the solutions of unheated soils and were more easily fermented. König, Coppenrath, and Hasenbäumer [e] found that heating soils with steam under pressure increased the solubility of both organic and inorganic matter. Other investigators who have shown that heating soils makes great changes in the soil constituents are Koch and Lüken,[f] Darbishire and Russell,[g] Pickering,[h] Schmoeger,[i] Russell and Hutchinson,[j] Lyon and Bizzell,[k] and Seaver and Clark.[l] In this laboratory, likewise, it has been found that dry and moist heat make great changes in the nature of the organic matter of the soil and in the productivity of the soil.

INCINERATION.

When various soils which had a strong oxidative power toward aloin were incinerated, the oxidative power was entirely lost.

ACIDS ON INCINERATED SOILS.

Acids like hydrochloric, sulphuric, acetic, citric, malic, and glycolic were then added to the incinerated soils and tests made again with aloin solutions. When 20 grams of the incinerated soils were treated with 12 c.c. of decinormal solutions of the acids and the soil then shaken with 50 c.c. of a 0.125 per cent water solution of aloin, the oxi-

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[a] Landw. Vers.-Stat., **47,** 269 (1896).
[b] Ann. Agron., **22,** 305 (1896).
[c] Landw. Vers.-Stat., **65,** 137 (1906–7).
[d] Arb. a. d. Kais. biol. Anstalt f. Land- und Forstwirt. Berlin, **5,** 126 (1906).
[e] Landw. Vers.-Stat., **63,** 471 (1906); **66,** 401 (1907).
[f] Jour. f. Landw., **55,** 161 (1907).
[g] Jour. Agr. Sci., **2,** 305 (1907).
[h] Jour. Agr. Sci., **2,** 411 (1908).
[i] Ber. deutsch. Chem., Ges., **26,** 386 (1893).
[j] Jour. Agr. Sci., **3,** 111 (1909).
[k] Bul. 275, Cornell Univ. Agr. Expt. Sta., 1910.
[l] Mycologia, **2,** 109 (1910).

dative power of the soils was renewed more or less, the oxidation being especially strong in the soils containing organic acids. In the case of the mineral acids the oxidation was very slight, being too slight to be compared with that produced by the addition of the organic acids. In the case of the limed soils the solutions were yellow. Accordingly, comparative readings were made in Hagerstown loam, untreated, plus complete fertilizer, and manured, incinerated, and treated with acetic, glycolic, malic, and citric acids. Taking the Hagerstown loam with acetic acid as 100, the comparative effect of the organic acids added to the incinerated soils is shown in Table VII.

TABLE VII.—*Effect of organic acids on the oxidative power of incinerated soils.*

Soils.	Acetic.	Glycolic.	Malic.	Citric.
Hagerstown loam, manured	100	184	180	223
Hagerstown loam, complete fertilizer	100	184	180	223
Hagerstown loam	100	172.5	192	238

It would seem from the preceding table that the organic hydroxyacids are more effective in restoring the oxidative function of incinerated soils than are nonhydroxyacids or the mineral acids. Of the hydroxyacids tried citric acid was the most effective in renewing the oxidative power of the incinerated soil. To test the activating action of the acids, they were added to soils which had been found to give little or no oxidation normally, such as Sassafras silt loam, Takoma lawn soil, Elkton silt loam, and Cecil sandy loam.

ACIDS ON NORMAL SOILS.

Addition of the acids, both inorganic and organic, to Elkton silt loam did not produce any oxidation of the aloin; added to Takoma lawn soil and Cecil sandy loam they produced a slight oxidation.

When 10 c. c. of decinormal acids were added to Sassafras silt loam, which has a slight oxidative power, hydrochloric acid retarded oxidation greatly, acetic acid produced less oxidation than the check, while malic, citric, and glycolic acids increased the oxidative power of the soil. With the untreated soils as 100, the relative oxidation was found to be: Soil plus acetic acid 73, soil plus glycolic acid 119, soil plus malic acid 106, soil plus citric acid 125. The relative increase in oxidative power produced by the hydroxyacids in different soils is not always the same, but in practically every case tried where treatment increases the oxidative power, the hydroxyacids increased it especially, while related nonhydroxyacids had little effect on oxidation. Thus acetic acid slightly retarded or but little increased oxidation, while the related hydroxyacid, glycolic, increased oxidation to a great degree; succinic acid had little effect on oxidation or retarded it, while the related hydroxyacids, malic and tartaric, greatly increased

oxidation; citraconic, itonic and mesaconic acids had a slight effect on oxidation in soils, while the related hydroxyacid, citric, greatly increased oxidation. Of the hydroxyacids, citric is somewhat the greater activator.

Sodium citrate and tartrate were next added to soils and their effect on oxidation compared with citric acid and tartaric acid. Five cubic centimeters of decinormal solution were added to 20 grams of soil, which was then shaken with 50 c. c. of 0.125 per cent solution of aloin. No increase of oxidation occurred in Takoma lawn soil or Cecil sandy loam. The salts, especially the citrate, effected changes in the soil so that it remained in suspension even after treatment with alcohol, centrifuging at high speed, and after hours of standing. In Sassafras silt loam the relative oxidation was: Untreated soil, 100; soil and sodium citrate, about 250; soil plus citric acid, 143; soil plus sodium tartrate, 111; soil plus tartaric acid, 118. In Hagerstown loam plus complete fertilizer the relative oxidation was: Soil untreated, 100; soil plus sodium citrate, 200; soil plus citric acid, 166; soil plus sodium tartrate, 154; soil plus tartaric acid, 118. The salts, sodium citrate and tartrate, were greater stimulators of oxidation than were equivalent quantities of free acids. The relative oxidation, however, is but an approximation, since the solution from the salt-treated soils could not be cleared up to get an accurate reading in the colorimeter. In a number of soils citric acid seemed to give a better oxidation than the sodium citrate, and in every case citric acid and citrate increased oxidation more than tartaric acid or sodium tartrate. Dihydroxystearic acid slightly reduced oxidation in soil, while the potassium and calcium salts slightly increased it.

When Takoma lawn soil and Elkton silt loam, which gave no oxidation directly and practically none with addition or inorganic and organic acids, were incinerated and treated with the acids, oxidation occurred in the soils treated with hydrochloric and glycolic acids and in no others. In the case of Takoma lawn soil hydrochloric acid gave an oxidation slightly greater than glycolic; in the case of Elkton silt loam the glycolic acid produced the greater oxidation. The incinerated Takoma lawn soil is the only soil in which treatment with hydrochloric acid produces an oxidizing power comparable to that produced by hydroxyacids. This exception, however, does not alter the fact that the hydroxyacids and their salts are especially favorably adapted for activating oxidative forces in soils.

Glycolic, malic, citric, and tartaric acids and sodium citrate and tartrate added to water solution of aloin produce no oxidation; added to pure quartz sand they have no oxidative power on aloin. Since these acids renew the oxidative power of incinerated soils, their activating action on the oxidative power of the soils must be due to their association with the inorganic constituents of the soil.

ACTION OF SALTS AND OTHER SUBSTANCES ON THE OXIDATION OF ALOIN.

Of the inorganic salts which may act directly upon aloin in the manner of an oxidase we have found the salts of ferric iron and of manganese the most effective. Ferric chloride solutions containing 3 parts of iron per million gave good oxidation of aloin. Ferrous chloride even in solutions containing 25 parts Fe per million did not oxidize aloin. With the addition of hydrogen peroxide, 1 part of iron per million as ferrous chloride or sulphate strongly oxidized the aloin. Manganese dioxide oxidized aloin readily, but less rapidly than ferric chloride. Other salts which have a slow but direct oxidizing action on aloin are calcium oxide, calcium carbonate, magnesium oxide, and manganese carbonate. Aluminum sulphate and chloride, containing 5 parts of aluminum per million, oxidize the aloin with the addition of hydrogen peroxide. Sodium and potassium hydroxide oxidize the aloin slowly. Copper sulphate plus sodium chloride oxidized the aloin solution immediately. On the organic side, benzaldehyde, quinone, piperidine, etc., oxidize aloin solutions readily. Decomposing cowpeas develop the power to oxidize aloin. This oxidizing power was not destroyed by ten minutes' heating in the water bath or by carbon bisulphide. Probably every autoxidizable body can activate the oxygen of the air and can thus oxidize other bodies.

In regard to the oxidative power of salts it may be said that the nature of the salts makes considerable difference. Thus manganese dioxide has a strong oxidizing power, while manganese chloride and sulphate have very little. Ferric hydroxide has very little oxidizing power, ferric citrate a slight oxidizing power, while ferric chloride oxidizes aloin very markedly. The addition of a small amount of hydrogen peroxide increases the oxidative action of the various salts as a rule. Citric acid and sodium citrate check the oxidative action of ferric chloride on aloin, but increase the oxidative action of manganese dioxide and manganese carbonate.

As previously shown, various salts of manganese, iron, aluminum, calcium, and magnesium have a greater or less oxidative action on a water solution of aloin. Accordingly these salts were added to soils to determine their action on the oxidative power of the soil to see whether they increased the oxidative action of a soil already possessing an oxidative power, or whether they generate an oxidative power in soils which have little of such a power. Their action was especially tested in conjunction with certain organic acids and salts, which, as already demonstrated, aid greatly in the oxidative processes.

INFLUENCE OF VARIOUS SALTS WITH AND WITHOUT CERTAIN ORGANIC ACIDS AND THEIR SALTS.

MANGANESE SALTS.

Soils may have practically the same amount of manganese and still vary greatly in oxidizing power, so oxidation in soils, if due to manganese, depends on the nature of the manganese as much as on the amount. The Hagerstown loam soils and Clarksville silt loam, however, which have a strong oxidizing power, contain considerable manganese. The Takoma lawn soil, Elkton silt loam, and Cecil sandy loam, which have very little oxidizing power, contain little manganese. Accordingly 100 parts of manganese per million in the form of manganese sulphate, chloride, carbonate, and manganese dioxide were added to the three soils which had been found poor in manganese. The soils were then shaken with aloin and in two or three hours the oxidizing power determined by means of the colorimeter. No increases in the power to oxidize aloin was noted in the case of the chloride, sulphate, or carbonate, in any of the soils. Manganese dioxide produced a slight oxidation in Takoma lawn and Cecil sandy loam and increased oxidation in Sassafras silt loam. When dilute acids such as citric, malic, and tartaric and salts such as sodium citrate and sodium tartrate were added to the soils to which the manganese had been added, there was a decided increase in the power to oxidize aloin. The best oxidation occurred in the soils to which manganese and citric acid had been added. With sodium citrate and tartrate the solutions, though showing good oxidation, were too turbid to read.

In order to study the action of the organic hydroxyacids on manganese without the disturbing influences which occur in the complex soil, manganese dioxide and manganese carbonate equivalent to 100 parts of manganese per million were added to 50 c.c. of 0.125 per cent water solution of aloin with and without citric and tartaric acids and sodium citrate and tartrate. In five hours the amount of oxidation was determined by means of the colorimeter. The relative oxidation was found to be manganese dioxide, 100; manganese dioxide plus 10 c.c. decinormal sodium citrate, 143; manganese dioxide plus 10 c.c. decinormal citric acid, 87; manganese carbonate plus 10 c.c. decinormal sodium citrate, 143; manganese carbonate plus 10 c.c. decinormal citric acid, no oxidation.

In a similar manner sodium tartrate greatly increased the oxidation of aloin by manganese dioxide. Tartaric acid in equivalent amounts at first increased the oxidative activity of manganese dioxide, but later retarded it. Dihydroxystearic acid decreased the oxidation by manganese dioxide as did the potassium and calcium salts.

Manganese sulphate and chloride solutions equivalent to 100 parts manganese per million have a faint oxidizing action on aloin in twenty hours. This oxidizing action is retarded by succinic, citric, and tartaric acids and slightly by sodium succinate. Sodium tartrate had little effect on the oxidative action of manganese sulphate and chloride, while sodium citrate increased the oxidation by these salts.

It would seem that, in contact with highly dilute manganese salt solutions, the salts of the hydroxyacids are in general the more effective activators. These results are in accord with those of Euler and Bolin,[a] who found that the neutral salts of aliphatic hydroxyacids, but especially sodium citrate and tartrate, quickened the oxidizing action of manganese acetate on hydroquinone, and in accord with their findings [b] that laccase of alfalfa consists of neutral salts, especially calcium, of one, two, and three basic hydroxyacids.

Citric, malic, and tartaric acids when added to the soil in the proportion of 0.0320 gram, 0.0325 gram, and 0.0375 gram, respectively, to 20 grams of soil, combine to a great extent with the bases present and increase the oxidative power of the soil in a way analogous to that in which the salts of these acids increase oxidation by manganese salts in solution.

FERRIC CHLORIDE.

Ferric chloride added to a water solution of aloin is a far more effective oxidizing agent than any salt of manganese. When added to soils of little or no oxidizing power, such as Takoma lawn, Elkton silt loam, Cecil sandy loam, Sassafras silt loam, ferric chloride did not bring about as much oxidation as manganese dioxide did, probably because the iron is reduced partly at least to the ferrous form, for, with the addition of hydrogen peroxide, the ferric chloride increased the oxidation. Ferric chloride produced some oxidation, however, in Takoma lawn soil and Cecil sandy loam and slightly increased it in Sassafras silt loam. In Elkton silt loam and Cecil sandy loam the further addition of 10 c.c. of citric acid and malic acid to 20 grams of soil retarded rather than aided oxidation. In Takoma lawn soil, the acids made a slight increase in oxidation. The salts of the acids gave solutions which were so turbid that they could not be read.

In the soils to which ferric chloride had been added the addition of 10 c.c. of decinormal hydrochloric acid favored oxidation, especially in the case of Takoma lawn soil and Cecil sandy loam. In Sassafras silt loam the organic acids, malic and citric, had the greatest favoring action on oxidation. The oxidation produced by the addition of ferric chloride was in no case as great as that brought about by the addition of manganese dioxide and the organic hydroxyacids

[a] Zeit. physiol. Chem., **57**, 80 (1908). [b] Zeit. phys. Chem., **69**, 187 (1909).

did not display so markedly the furthering action on oxidation so frequently spoken of. Ferric oxide equivalent to 100 parts of ferric iron per million added to Sassafras silt loam and Takoma lawn soil slightly increased the oxidizing power of the soils, especially with the further addition of 10 c.c. of decinormal hydroxyorganic acids. But the increase in oxidizing power effected by 100 parts of iron per million was by no means so great as that effected by 100 parts of manganese per million.

ALUMINUM SALTS.

The addition of aluminum oxide and aluminum hydroxide to soils increased the oxidizing power little if any. When citric, malic, and tartaric acids were added in addition to the aluminum, there was considerable increase in the oxidizing power in every soil tested, though not to such an extent as when manganese dioxide was added. Hydrochloric acid tended to decrease the oxidation.

CALCIUM SALTS.

Calcium carbonate increased the oxidizing power of soils in the presence of the organic hydroxyacids. Here, again, hydrochloric acid brought about no increased oxidation.

MAGNESIUM SALTS.

The addition of magnesium carbonate and organic hydroxyacids such as citric, malic, and tartaric increased oxidation in soils which showed little or no oxidation per se or with the addition of magnesium carbonate alone and increased the oxidation in soils which normally gave oxidation. Hydrochloric acid was of little influence in this regard.

The experiments show that inorganic salts in the presence of certain organic hydroxyacids and salts of these acids can act as carriers of oxygen to easily oxidizable bodies. Of the organic hydroxyacids tested (glycolic, malic, tartaric, and citric), citric proved to be the best activator of the inorganic soil constituents, and sodium citrate was better still. Acetic, succinic, and hydrochloric acids had practically no activating effect. Of the inorganic substances studied, manganese was found to favor oxidation in soils to the greatest extent in the presence of hydroxyacids.

The part that inorganic salts may play in the oxidative phenomena of living things was noted by Bertrand. He studied in detail the oxidizing principle discovered by Yoshida[a] in the juice of the lac tree, *Rhus vernicifera*, and designated it as an enzyme, laccase. Bertrand[b] found that laccase contained manganese and that this metal played

[a] Jour. Chem. Soc., **43**, 472 (1883.) Bertrand, Ann. Chem. Phys. **12**, 115 (1897).
[b] Bul. Soc. Chim., **13**, 361 (1895); **17**, 619; (1897).

an important rôle in the oxidation by laccase. According to Bertrand the Rhus laccase consists of an organic portion and manganese. The organic portion Bertrand believes he has found in other plants, as *Medicago sativa* and *Lolium perenne*, and he speaks of these preparations as laccase.[a] Bertrand[b] considers that laccase like all diastases is composed of two complementary elements: (1) An active complement; (2) an activating complement. In general the active complement is an acid or alkaline substance or a salt of calcium, manganese, etc. The activating complement is a more complex substance analogous to egg albumin and is thermolabile. As regards laccase, the active complement is a salt of manganese, the activating complement, thermolabile complex organic matter. In the course of his investigations Bertrand found that the oxidizing power of laccase was proportional to the amount of manganese present and that oxidations accomplished by laccase are greatly accelerated by small amounts of manganese salts and that no other metal is capable of accelerating the oxidation brought about by laccase. Euler and Bolin[c] found that the laccase or activating principle of *Medicago sativa* consisted of a mixture of neutral salts, especially calcium, of one, two, and three basic hydroxyacids. Among these acids they found citric, malic, mesoxalic, and glycolic. The neutral salts of these acids they found exercised the same oxidative action in connection with manganese as Medicago laccase did. They regard the oxidizing principle of Medicago laccase as nonenzymotic.

Whether or not the oxidizing principle in the soil is manganese or other salts which are activated by the organic hydroxyacids in the incinerated soil and are rendered more effective in the normal soil, it seems most probable that the oxidizing power of the soils is due not to enzymes, but to a combination of inorganic constituents with definite kinds of organic matter. Since true enzymes readily undergo decomposition in solution, it should be expected that they would undergo decomposition readily in the soil and would exist only temporarily under ordinary soil conditions.

FUNCTION OF MANGANESE AS A FERTILIZER.

It has long been known that manganese exists in many plants. According to Bertrand,[d] Scheele[e] made the first observation on this subject. He found manganese to a small extent in the ash of wild anise and to a greater extent in the ash of wood. Later, in 1849, according to Bertrand, Herapath found manganese in the ash of radish, beet, and carrot, while Richardson noted its presence in the

[a] Bul. Soc. Chim., **17**, 621 (1897).
[b] Revue scientifique, 1906, 609.
[c] Zeit. phys. Chem., **69**, 187 (1909).
[d] Rev. gén. de chimie, **8**, 205 (1905).
[e] Mémoires de Chymie Dijon, 1785.

ash of cane sugar. Salm-Hortsmar[a] found it in oats and employed manganese as a nutrient in culture experiments in carbon made from cane sugar. In these experiments he found manganese stimulated growth and increased the assimilation of other salts present in nutrient solution added to the carbon. Sachs[b] attempted to substitute manganese for iron in nutrient solution and found that the leaves became yellow and etiolated.

In 1872 LeClerc[c] by a delicate colorimetric method found manganese in most of the soils and vegetables examined by him. Maumené[d] found manganese in many vegetables, but especially in wheat as a salt of an organic acid. Pichard[e] and Gössl[f] found it widespread in plants and animals.

From the time that Bertrand[g] showed that manganese played an essential part in the oxidation by the so-called oxidizing enzyme, laccase, and suggested the study of manganese as a fertilizer, considerable work has been done on the salts of manganese as a fertilizer. This work is well reviewed by Giglioli[h] and Rousset.[i]

Giglioli[j] applied manganese dioxide to soils cultivated with wheat and corn and found that the yields were increased.

Loew and Sawa[k] studied the effect of manganese sulphate on cultures of barley, wheat, peas, radishes, cabbage, etc., in solution and in pots and found vegetation was increased when the manganese did not exceed 0.02 per cent. An excess of manganese exerted an injurious action consisting in the bleaching out of the chlorophyll. They found that juices of bleached plants had a more intense oxidative power than the juices from healthy plants.

Voelcher[l] experimented with manganese in pot cultures of wheat and barley. The yield of wheat was increased by the sulphate, chloride, phosphate, and dioxide of manganese, the nitrate increased the yield of straw, but decreased the grain. In the case of barley, the oxide of manganese decreased the yield slightly, while the other manganese salts increased the yields.

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[a] Jour. prakt. chem., **46–47,** 193 (1849).

[b] Hofmeister's Handbuch. Phys. Botanik., **4,** 144 (1865).

[c] Compt. Rend., **75,** 1209 (1872).

[d] Compt. Rend., **98,** 1416 (1884).

[e] Compt. Rend., **126,** 1882 (1898).

[f] Bot. Centr., Beiheft., **18,** 119 (1905).

[g] Compt. Rend., **122,** 1132 (1896); **124,** 1032 (1897); Bul. Soc. Chim., **17,** 619 (1897).

[h] Boll. quindicinale della Soc. degli. Agr. Ital., **13,** 974 (1908).

[i] Ann. Sci. Agron., **2,** 81 (1909).

[j] Ann. di Scuola sup. d. agr. di Portici, 1901. Referred to in Boll. quindicinale della Soc. degli Agr. Ital., **13,** 974 (1908).

[k] Bul. Col. Agr. Tokyo, **5,** 161 (1902–3).

[l] Jour. Roy. Agr. Soc., **64,** 348 (1903).

Nagaoka[a] found manganese sulphate stimulated growth of rice and that the beneficial effect of the manganese was evident in the following year, the yield being 16.9 per cent greater than in the soils not fertilized with manganese. Aso[b] found that the cheaper manganese chloride increased the yield of rice one-third. Fukutome[c] found that the joint action of ferrous sulphate and manganese chloride had a marked effect on the growth of flax, while each alone had but little effect. Bertrand and Thomassin[d] found that fertilizing soil with manganese sulphate increased the yield of oats 22.5 per cent. Salomone[e] found that manganese sulphate in moderate amounts favored the growth of wheat. Moliari and Ligot[f] cultivated oats in pots containing 0.01 to 0.07 per cent of manganese in the form of sulphate with and without other fertilizers. Fertilizing with manganese considerably increased the yield of straw and grain. Other workers who have found that small applications of manganese to soils stimulated growth are Grégoire, Hendrick, and Corpiaux;[g] Garola,[h] who found that manganese salts greatly increased growth of flax and caused a greater assimilation of the fertilizing elements nitrates, phosphoric acid, potassium, calcium, manganese, etc.; Haffner[i] who found that 50 kilograms of manganese sulphate to the hectare increased the yield of rice from 26 to 75 per cent. Sjollema and Hudig[j] found that oat-sick soils could be restored to health by the application of manganese sulphate.

Excess of manganese in soils may prove injurious, as shown by the work of Loew[k] on rice, peas, and cabbage; of Salomone[l] on wheat; by the investigation of Kelley[m] on Hawaiian pineapple soil and of Guthrie and Cohen[n] on the failure of grass on Australian soils.

From the evidence at hand it seems that in most cases manganese in moderate quantity can exercise a useful stimulating action on plant growth. Investigations by Javillier, Lecarme, and others[o] point out that manganese salts should be used in small amounts, 8.9 to 35.7

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[a] Bul. Col. Agr. Tokyo, **5,** 467 (1902–3); **6,** 135 (1904–5).
[b] Bul. Col. Agr. Tokyo, **6,** 131 (1904–5).
[c] Bul. Col. Agr. Tokyo, **6,** 137 (1904–5).
[d] Compt. Rend., **141,** 1255 (1905).
[e] Le Staz. Sper. Agr. Ital., **38,** 1015 (1905); **40,** 97 (1907).
[f] Bul. du Ministère de l'Agr. de Belg., **23,** 764 (1907).
[g] Bul. du Ministère de l'Agr. Belg., **23,** 388 (1907).
[h] Referred to by Rousset, loc. cit.
[i] Bul. Econ., **11,** 514 (1908).
[j] Verlag Landbouwk Onderzoek Rijkslandbouwproefstat., Netherlands, **5,** 29 (1909). E. S. R., **21,** 115 (1909).
[k] Loc. cit.
[l] Le Staz. Sper. Agr. Ital., **38,** 1015 (1905); **40,** 97 (1907).
[m] Hawaii Sta. Press Bul., 23, Jour. Indust. and Eng. Chem., **1,** 533 (1909).
[n] The Agricultural Gazette of New South Wales, **21,** 219 (1910).
[o] See Mark Lane Express, **100,** 305 (1909). Le Phosphate, **18,** 111 (1909.)

pounds of manganese to the acre, pulverized and mixed with chemicals or barnyard manure.

Since manganese salts added to soils increased the oxidative power of the soil more than other salts do and manganese added to soil solutions increase the oxidative power of wheat plants grown therein, it would seem that manganese plays its part as stimulator of growth by its action in furthering oxidation, both in the soil and in the plant. In previous work[a] it has been shown that whatever increased the oxidative power of the plant roots tends to increase the growth of the plant and with increased growth there is generally an increased oxidative power in the roots, and vice versa, that whatever retards oxidation by the plant roots tends to retard the development of the plant. In previous work[b] it has been shown that many soils are infertile not because they are lacking in plant food, but rather because they contain organic substances injurious to plant growth. Soils containing such injurious substances have been found to improve in productivity by keeping in the laboratory, either under the action of microorganisms or because they undergo spontaneous or autoxidation with the formation of noninjurious bodies. In passing, it may be said that whatever changes these injurious substances should favor the productiveness of the soil. The favorable effect of steaming soils may be explained in part by the destructive action of the steam on the injurious bodies.

Tyrosine, a body arising in the digestion of protein, whether by the proteolytic enzymes of animal, microorganism, or plant, is toxic to wheat. A tyrosine solution in contact with air spontaneously oxidizes and becomes red or even black, depending on the degree of oxidation. When thus oxidized, the tyrosine solution instead of being harmful to plants is decidedly beneficial. In a similar manner, the water extract of green manure, such as cowpea and the water extract of wheat seedlings are injurious to plants when fresh, but on oxidizing and darkening the extracts become decidedly beneficial. Bodies which are naturally toxic to plants, such as vanillin, have been found to be oxidized by growing plant roots, especially in the presence of sodium nitrate and calcium carbonate,[c] substances which have also been found capable of increasing the oxidizing power of soils. It has been found in addition that growing crops[d] leave material in the soil which is harmful to succeeding crops, either directly or after secondary decomposition, and which is overcome by thorough oxidation.

The stimulating action of manganese may be found most probably in its oxidizing activity and its effect on the oxidizing power of plant,

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[a] Bul. 56, Bureau of Soils, U. S. Dept. Agr.

[b] Buls. 36 and 53, Bureau of Soils, U. S. Dept. Agr. Jour. Biol. Chem., **6**, 39 (1909).

[c] Bul. 47, Bureau of Soils, U. S. Dept. Agr.

[d] Buls. 36 and 40 Bureau of Soils, U. S. Dept. Agr.; Jour. Biol. Chem., **6**, 39 (1909).

microorganism, and soil. In connection with plants, Bertrand[a] has shown it to be the most active element in promoting oxidation changes. In soils we have found it capable of promoting the most active oxidation, especially in the presence of suitable organic matter, such as salts of organic hydroxyacids. By its strong oxidizing power manganese would render the injurious material in the soil harmless or even beneficial and by the oxidation of inert or rather stable organic matter might cause the nitrogen and other stubstances contained in the organic matter to become more rapidly available to plants. That manganese is acting on the soil ingredients as a catalyzer or oxidizer seems evident from the results obtained by Bertrand[b] wherein he found that plants which were greatly accelerated in growth by fertilizing with manganese contained no more manganese than the plants in soils not so fertilized. According to Giglioli[c] the presence of compounds which accumulate combined oxygen with a capacity of giving it off gradually, facilitate the deep development of roots and render them capable of developing in the deeper strata of the soil which otherwise they would not be able to penetrate. When a ditch is dug in a region where manganese dioxide is acting as an oxidizer, a deep system of roots is found. By bringing about greater oxidation and better growth, such compounds as manganese dioxide would aid in establishing better drainage in soils.

EFFECT OF CROPPING AND FERTILIZING ON SOIL OXIDATION.

Wheat was planted in Hagerstown loam untreated, Hagerstown loam manured, and Sassafras silt loam, as examples of soil which had shown oxidizing power readily, and Takoma lawn soil, Arlington clay loam, and Cecil fine sandy loam, as examples of soil with poor oxidative power. The seedlings were allowed to grow seventeen days and the green weight was taken. Three days later the soils were sifted and the oxidative power of the soils was compared by shaking 20 grams of each soil with 50 c. c. of the 0.125 per cent aloin and comparing it with the unplanted air-dried laboratory samples. The results are shown in Table VIII.

TABLE VIII.—*Oxidation in cropped soil as compared with air-dried samples unplanted.*

Soil.	Green weight, 12 plants.	Oxidation.	
		Unplanted.	Planted.
	Grams.		
Hagerstown loam, manured	2.915	100	165
Hagerstown loam	2.420	100	133
Sassafras silt loam	3.835	100	175
Takoma lawn soil	2.275	Neg.	Slight.
Arlington clay loam	2.510	Slight.	Slight.
Cecil fine sandy loam	1.930	Neg.	Faint

[a] Loc. cit. [b] Comp. Rend., 141, 1255 (1905). [c] Loc. cit.

The growth of the wheat seedlings in the soils increased the oxidative power. It was probably not possible, however, to separate the soil absolutely from the root débris and the increased oxidizing power of the planted soil may come partly from the oxidizing power of the root cells contained therein or from the oxidizing enzymes left in the soil during the growth of the root. That the increased oxidation in the planted soil is due to the oxidizing powers of the root débris may be seen in the fact that in the planted soils as in the case of oxidation by the intact root, the oxidation is somewhat gradual, being greater five hours after shaking with aloin than in two hours after shaking, whereas the oxidation by the unplanted soil remains the same whether determined in two or five hours after contact with the aloin solution. It would seem then that the oxidation of cultivated soil may be due to the oxidative powers of the soil constituents and to the material left in the soil during the growth of plants and perhaps by micro-organisms.

The effect of cropping on the oxidative power of the soil without the addition of fertilizers is variable. In some soils cropping increased the oxidative power of the soils; in others it decreased it slightly. Rarely, however, was cropping without some effect on the oxidative power of the soil.

The oxidative power of Arlington clay loam fertilized with sodium nitrate, potassium sulphate, and calcium phosphate, and mixtures of these salts in the proportion of 50 pounds of NH_3, K_2O, and P_2O_5 and mixtures of NH_3, K_2O, and P_2O_5 to the acre upon which wheat had grown twenty-three days, May 14 to June 6, was next determined. The planted soils gave a turbid solution and a shade of oxidized aloin so different from the same soil unplanted that the planted and unplanted soil could not be compared. The planted soils, however, were comparable. The results are shown in Table IX.

TABLE IX.—*Oxidation in Arlington clay loam upon which wheat had grown.*

Treatment.	Green weight, 18 plants.	Relative oxidation.
	Grams.	
Soil unfertilized	2.203	100
Soil plus sodium nitrate	3.324	176
Soil plus potassium sulphate	2.600	150
Soil plus acid calcium phosphate	2.702	115
Soil plus sodium nitrate, potassium sulphate, acid calcium phosphate	3.228	158

Seventeen days later, June 23, the oxidative power of the Arlington soil was again tested. All the samples were air dried. The planted soils no longer gave a turbid solution and the shade of the oxidized aloin of the planted and unplanted check were quite comparable. The relative oxidations are shown in Table X.

TABLE X.—*Oxidation in Arlington clay loam upon which wheat had grown and which had air-dried in the laboratory.*

Treatment.	Relative oxidation.
Soil unfertilized and unplanted	100
Soil unfertilized and planted	96
Soil plus sodium nitrate, planted	132
Soil plus potassium sulphate, planted	112
Soil plus acid calcium phosphate, planted	122
Soil plus sodium nitrate, potassium sulphate, acid calcium phosphate, planted	128

The oxidative power of Orangeburg loam from Alabama on which wheat had grown for twenty-one days was next tested; first, immediately after the wheat had been cut off, and again after the soils had stood in the laboratory for nine days. When first tested, the planted soil gave a turbid solution which could not be compared with the unplanted soil solution. In the second testing the solutions obtained by shaking the aloin solution with the soil was quite clear and comparable throughout. In contact with Orangeburg loam the oxidized aloin is made yellow again in a few hours, so the oxidation test ran only about twenty-five minutes. The relative oxidations are given in Table XI.

TABLE XI.—*Oxidation in Orangeburg loam upon which wheat had grown. Tested immediately after growth of wheat.*

Treatment.	Relative oxidation.
Soil unfertilized	100
Soil plus sodium nitrate	100
Soil plus potassium sulphate	94
Soil plus acid calcium phosphate	100
Soil plus sodium nitrate, potassium sulphate, acid calcium phosphate	103

Table XII shows the oxidation in the soils nine days later:

TABLE XII.—*Oxidation in Orangeburg loam on which wheat had grown. Air-dried in Laboratory.*

Treatment.	Green weight.	Relative oxidation.
	Grams.	
Soil unfertilized, unplanted	1.829	100
Soil unfertilized, planted	1.829	98
Soil plus sodium nitrate, planted	2.744	125
Soil plus potassium sulphate, planted	1.746	113
Soil plus acid calcium phosphate, planted	1.832	114
Soil plus sodium nitrate, potassium sulphate, acid calcium phosphate, planted	2.080	121

The oxidative power of Volusia silt loam from Ontario County, N. Y., was next tested twenty-four hours after cutting off wheat which had grown thereon fifteen days. The results are given in Table XIII.

TABLE XIII.—*Oxidation in Volusia silt loam, planted and unplanted.*

Treatment.	Green weight.	Relative oxidation.
	Grams.	
Soil unfertilized, unplanted		100
Soil unfertilized, planted	0.430	92
Soil plus sodium nitrate, planted	.900	136
Soil plus potassium sulphate, planted	.408	100
Soil plus acid calcium phosphate, planted	.400	100
Soil plus sodium nitrate, potassium sulphate, acid calcium phosphate, planted	.670	107

It would seem from the tables that the fertilizers may increase the oxidative power of planted soils.

EFFECT OF FERTILIZER SALTS ON SOIL OXIDATION.

To determine the effect of fertilizer salts on the oxidative power of the soils without the presence of plants, 50 grams of air-dried soil were treated with sodium nitrate, potassium sulphate, acid calcium phosphate, and mixtures of equal parts of these salts so that each soil contained the equivalent of 50 parts of NH_3, K_2O, and P_2O_5 per million, respectively, and mixtures of NH_3, K_2O, and P_2O_5. The soils were brought to optimum moisture content and were tested twenty-four and seventy-two hours later with 60 c. c. of a 0.125 per cent aloin solution. Each soil was shaken with the aloin solution for two minutes and then allowed to settle. After thirty to forty-five minutes, the solutions were filtered, in some cases after the addition of alcohol, the filtrate centrifuged, and the depth of color determined by means of a colorimeter. The results are given in Table XIV.

TABLE XIV.—*Effect of fertilizers on oxidation in soils.*

Soil.	Relative oxidation.				
	Untreated.	Plus sodium nitrate.	Plus potassium sulphate.	Plus acid calcium phosphate.	Plus sodium nitrate, potassium sulphate, and acid calcium phosphate.
Sassafras silt loam (a, b)	100	[a] 136 [b] 166	142 150	150 156	142 156
Arlington clay loam (a, b)	100	135 120	142 115	122.5 125	126 125
Clarksville loam (a)	100	118	133	154	148
Hagerstown loam	[b] 100	143	154	143	143
Volusia silt loam (a, b)	Neg.	Neg.	Neg.	Neg.	Neg.
Dutchess silt loam (a, b)	Neg.	Neg.	Neg.	Neg.	Neg.
Dekalb silt loam (a, b)	Neg.	Neg.	Neg.	Neg.	Neg.
Norfolk fine sandy loam (a, b)	Neg.	Neg.	Neg.	Neg.	Neg.

[a] Oxidation tested in twenty-four hours after adding the fertilizers.
[b] Oxidation tested in seventy-two hours after adding the fertilizers.

In another experiment 100 grams of each soil, which had been kept at optimum moisture, were treated with sodium nitrate, potassium sulphate, acid calcium phosphate, and mixtures of equal parts

of these, so that each soil contained the equivalent of 200 parts of NH_3, K_2O, and P_2O_5 per million. Five days later the soils were tested for their oxidative power, with the results shown in Table XV.

TABLE XV.—*Effect of fertilizers on oxidation in soils.*

Soil.	Relative oxidation				
	Check.	Plus sodium nitrate.	Plus potassium sulphate.	Plus acid calcium phosphate.	Plus sodium nitrate, potassium sulphate, and acid calcium phosphate.
Takoma lawn soil	Neg.	Neg.	Neg.	Neg.	Neg.
Cecil sandy loam	Neg.	Neg.	Neg.	Neg.	Neg.
Elkton silt loam	Neg.	Neg.	Neg.	Neg.	Neg.
Orangeburg loam	100	(a)	94	143	100
Clarksville loam	100	86	95	100	86
Sassafras silt loam	100	86	97	91	95
Hagerstown loam	100	81	94	103	88
Arlington clay loam	100	74	83	86	86

[a] Turbid. Good oxidation.

Numerous experiments have been made on the effect of fertilizers on the oxidative power of soils with results that show that the fertilizers sometimes increase oxidation and sometimes decrease it, and that the effects of the fertilizers are different in the different soils.

The action of the fertilizing salts must be attributed to the effect they have on the microorganisms of the soil, modifying their numbers and activities with a resulting modification of biochemical activities and a resulting change in both the amount and the condition of inorganic and organic soil constituents, or directly on the soil constituents, especially on the organic matter which is undergoing changes by autoxidation or as a result of the activity of microorganisms. In short the fertilizers added to soil or present in soils do work and bring about various changes, one of which is made manifest by the changed oxidative power.

That fertilizers do work in the soil even before planting is indicated by experiments on the time action of fertilizers. One hundred parts per million of sodium nitrate were added to a soil which was known to be benefited by such application and an aqueous extract prepared after the nitrate had been added thirteen days. Similarly, sodium nitrate was added to another portion of the same soil and an aqueous extract prepared immediately. Wheat seedlings were then grown in the two extracts. The results showed that the growth of the plants in the extract from the soil to which the sodium nitrate had been added thirteen days earlier was far better than the growth in the extract made from the soil immediately after adding the nitrate, although analysis showed the nitrate to be higher in the latter extract. An experiment on the time action of fertilizers on the soil before planting

was also made on the Arlington clay loam, a soil responding to nitrate fertilization. This experiment was made by adding sodium nitrate, potassium sulphate, and acid calcium phosphate at intervals of one week and changing the order of their addition. The soils with fertilizer addition were kept at optimum moisture condition and at the end of the third week planted with wheat. Without dwelling on the results in detail it may be said that the various time actions of the potassium sulphate and monocalcium phosphate seemed to produce no noticeable effect on the growth of the plants in this soil, but with the time action of the sodium nitrate the effect was very striking. The sodium nitrate added at the time of planting produced an increase in the green growth of wheat amounting to 24 per cent; applied one week before planting the increase was 33 per cent; applied two weeks before planting the increase was 36 per cent; and applied three weeks before planting the increase was 44 per cent. The series of yields show a regular increase according to the length of time that the sodium nitrate was acting on the soil before planting.

OXIDATION AND STIMULATING ACTION OF SALTS ON GROWTH.

The stimulating action of the salts on growth, such as nitrates, phosphates, potassium, salts of calcium and magnesium, especially the oxides and carbonates, salts of iron and aluminum, especially the higher oxides, may be partly, at least, explained on their capacity for effecting oxidation and reduction processes in soils, in stimulating the activities of microorganisms, thus changing injurious compounds to harmless or beneficial compounds and rendering the ingredients of inert, immobile organic material available to plants. In a general way lime has long been known to promote oxidation.

Recent researches [a] have shown that the nature of the organic matter of the soil may be in certain cases the limiting factor in soil fertility, so that it is not improbable that the beneficial results of fallowing are partly to be found in the proper changes of organic matter in the soil, either by oxidation or by the decomposing action of microorganisms.

THE RELATION OF ORGANIC MATTER TO SOIL OXIDATION.

Oxidation in soils is undoubtedly due to various factors and is dependent not only on the inorganic constituents of the soil, but also on the organic matter. In the case of the Hagerstown loam the manured soil had by far the best oxidizing power. With the unfertilized soil as 100, the plot which had received 10 tons of stable manure to the acre was 136 and over. Decomposing cowpea vines added to Sassafras silt loam increased the oxidizing power. When various

[a] Buls. 36, 40, 47, and 53, Bureau of Soils, U. S. Dept. Agr.

soils were steam heated or heated at 105° C. for an hour, the oxidative power was greatly lessened. This decrease in the oxidizing power seems to be due mainly to changes in the organic matter. When the organic matter of the soils was destroyed by incineration, the oxidizing power was lost. The oxidative power of the soil is dependent on the nature of the organic matter. Thus, when salts of manganese, iron, calcium, magnesium, and aluminum were added to soil of slight oxidative power, oxidation was but slightly increased until certain kinds of organic matter were added, such as citric, malic, tartaric, glycolic acids, or their salts. These compounds we found had a reducing action, converting ferric compounds to ferrous compounds and probably bringing about the stimulation of the oxidative power of the bases mentioned by means of a preliminary reduction to lower, less oxygenated compounds, which, in turn reoxidizing themselves, activate the oxygen of the air. This active oxygen oxidizes other bodies, such as organic material of the soil or aloin, which is employed as a test of oxidation. It may be, too, that hydroxyacids affect the oxidative powers of complex organic substances or of inorganic-organic complexes.

It is possible that hydroxyacids, like citric and the salts of these, are formed in soils. Wehmer[a] has shown that certain molds, to which he gave the name of citromycetes, form citric acid from carbohydrates. The spores of these molds he found widespread in air. Maze and Perrier[b] found that citric acid was formed from sugars, glycerin, and alcohol. Sullivan[c] found that certain bacteria formed citric acid during pigment formation. Compounds of a nature similar to hydroxyacids and their salts, but of a more complex nature, undoubtedly exist in soils and by their properties facilitate soil oxidation. Some highly oxygenated compounds have been isolated from soils.[d] Dihydroxystearic acid isolated from a number of soils behaves differently from simpler, more soluble hydroxyacids in that it retards oxidation both in solution and in soil. The calcium and potassium salts, on the other hand, have a slight accelerating effect on oxidation in soils.

Fresh plant juices have the power to oxidize aloin directly. On standing, this direct oxidizing power is often lost, either because of change in the reaction of the juices or because the oxidizing principles have undergone changes. After the loss of the direct oxidizing power, the plant juices, as a rule, have an indirect oxidizing power; that is, they can oxidize with the addition of hydrogen peroxide.

The addition of hydrogen peroxide increased the oxidizing action of some soils, decreased it in others. The peroxide increased the oxidative power of Takoma lawn soil and Cecil fine sandy loam,

[a] Bul. Soc. Chim., **9**, 728 (1893).
[b] Ann. Inst. Pasteur, **18**, 553 (1904).
[c] C. f. B., II, **15**, 243 (1905).
[d] Bul. 53, Bureau of Soils, U. S. Dept. Agr.

which give no direct oxidation of aloin, and decreased oxidation in Sassafras silt loam and Hagerstown loam, manured, which normally oxidize aloin directly. It is possible that the soils which normally give a direct oxidation of aloin contain both organic and inorganic peroxides. It is highly probable that soils rich in organic matter would contain organic peroxides, since such soils have been found by us to have the ability to absorb oxygen. Decomposing cowpea was found to have a strong oxidizing power on aloin—an oxidizing power which was not entirely destroyed by heating ten minutes on a steam bath or by carbon bisulphide. That organic bodies will form peroxides in the presence of air is shown by Baeyer and Villiger,[a] who showed that when benzaldehyde is oxidized by atmospheric oxygen, benzoyl peroxide is formed, $C_6H_5CHO + O_2 = C_6H_5COOOH$, and by Ditz,[b] who found that ethyl ether in contact with air forms within itself more or less ethyl peroxide. This ethyl peroxide he found had a stronger oxidizing action than hydrogen peroxide.

Bach[c] obtained the characteristic reaction for peroxide with titanium sulphate, when the following substances acted on air for some time: Nascent hydrogen, phosphorus, sodium, potassium, lead, methyl alcohol, ethyl alcohol, glycerine, formaldehyde, acetic aldehyde, benzoic aldehyde, acetic, oxalic, and tartaric acids, phenol, resorcin, pyrocatechin, tannin, pyrogallol, phenylhydrazine, petroleum, quinone, etc. By analogy Bach concluded that the so-called oxidizing ferments in blood are simply readily oxidizable substances having a special aptitude for forming peroxides which oxidize other substances more difficult to oxidize.

As previously pointed out, Kastle and Loevenhart[d] in discussing the nature of the oxidizing ferment of the potato conclude that the so-called oxidizing ferment is in all probability not a true ferment, but an organic peroxide. They further conclude that oxidation phenomena occurring in plants and probably also in animals can be satisfactorily explained upon the supposition that the readily autoxidizable substances which they contain are oxidized to the peroxide condition by molecular oxygen and that the peroxides thus formed in turn give up part, if not all, of their oxygen to other less oxidizable substances present in the cell. In other words, the process of rendering oxygen active by the living cell is probably brought about in essentially the same way that this is accomplished by phosphorus, benzaldehyde, and other oxygen carriers; that is, it is one phase of autoxidation.

Peroxide forming bodies such as benzaldehyde and peroxides like quinone have a strong oxidizing action on aloin, both in solution and in soil.

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[a] B. deutsch Chem. Ges., **33,** 1569 (1900).
[b] Chem. Zeitg., **29,** 705 (1905).
[c] Compt. Rend., **124,** 951 (1897).
[d] Am. Chem. Jour., **26,** 539 (1901).

It is possible that in the soil complex autoxidizable substances are formed in the changes brought about by the action of microorganisms on plant débris, etc. These autoxidizable substances would combine with the oxygen of the air in much the same way that benzaldehyde does and would form complex, more or less unstable, peroxides, which in turn would give up a part or all of their oxygen to oxidizable substances in contact with them or would further oxidize themselves.

The possibility of the formation of peroxides in soil even to excess is indicated by Sjollema and Hudig,[a] who found that such soils were restored to a good condition by manganese sulphate, and suggested that oat sickness may be due to the formation in the soil in the presence of excess of calcareous fertilizers and physiological alkaline fertilizers, as nitrate of soda, of large amounts of peroxides injurious to plants. The favorable action of the manganese sulphate is due, according to these investigators, to the catalytic decomposition of these injurious peroxides.

THE EFFECT OF EXCESSIVE OXIDATION.

Whatever induces excessive oxidation in soil or plant would undoubtedly be injurious. Thus Woods[b] found that excessive oxidizing enzymes in plants destroyed the chlorophyll. Loew and Sawa and Salomone[b] found that an excess of manganese sulphate added to soil decreased the growth of plants with a great increase of the oxidative power of the plant juices, and according to Kastle and Elvove[c] strong oxidizing agents like nitrates, nitrobenzene, picric acid, chromates, chlorates, arsenates, organic peroxides, and peracids are poisonous to life. In small amounts these substances may be stimulative to life functions. In a similar manner, quinone, a strong oxidizer, is poisonous to plants such as wheat. Under normal conditions, as in the plant, the excessive formation of peroxide is prevented in some way, either by the further oxidation of the peroxides or by the catalytic or peroxide splitting power of the soils. Most soils have this catalytic power to a greater or less degree, and in general we have found that fertile soils have a greater catalytic power as well as a greater oxidative power than infertile soils. The relation between oxidation and catalysis is not as clear as it might be, even in the plant where it has been extensively studied.

According to Chodat[d] the only property of the catalase of which we have certain knowledge is its power to decompose hydrogen per-

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[a] Loc. cit.

[b] Loc. cit. In connection with excess of manganese in soil and soil oxidation see also Kelley and Guthrie and Cohen (loc. cit.).

[c] Am. Chem. Jour., **31,** 195 (1904).

[d] Schweitz. Wochenschr. Chem. u. Pharm., **43,** 626, 642, 655 (1905).

oxide to water and molecular oxygen. According to Loew,[a] Herlitzka,[b] Battelli and Stearn,[c] and Shaffer,[d] on the other hand, the catalase of plants has a protective influence against excessive oxidation. The catalytic power of the soil may in time be shown to have a similar action in promoting normal oxidation in soil, and like oxidation may be connected with soil conditions adapted to the growth of the majority of plants.

BIOLOGICAL ANALOGIES IN SOIL OXIDATION.

Whether oxidation in plants is due to enzymes or not is still debatable. Oxidation by soil seems to be due mainly to non-enzymotic forces. The analogies between the oxidation by soils and that by plants and animals are quite close, however, and perhaps it would be well to bring out these analogies here by making a brief review of oxidation from an historical side with some remarks on the present status of our knowledge of oxidation by presumably enzymotic means.

From the time of the discovery of oxygen by Priestley in 1774, and the researches of Lavoissier on combustion (1774–1785) to the discovery of ozone by Schönbein in 1840, many observations had accumulated in the literature concerning the influence of one substance on the oxidation of another by air or oxygen. The historical development of the theories of oxidation is well brought out by Kastle.[e] The first study of oxidation by living tissue is that made by Planche.[f] He found that fresh roots of horse-radish had the power of turning tincture of guaiac blue. In 1819 Taddey[g] found that wheat flour blued guaiac and in 1820[h] tested the behavior of a large number of substances toward guaiac, among them roots, fresh and dried, gums, milk, soap, etc., and found that the substances on heating lost the power to blue guaiac. Schönbein,[i] the discoverer of ozone, studied the bluing of guaiac in detail. He showed that guaiac is colored blue by ozone[j] and concluded that the blue material resulting from the action of ozone on guaiac is a compound of ordinary guaiac with hydrogen peroxide. In other words, that it possesses the nature of an organic peroxide which contains at least a part of its oxygen in

[a] Report 68, U. S. Dept. Agr. (1901).

[b] Rend. Sci. Fis. Mat. et Nat. Real. Acad. Lincei., **16,** ser. 5, part 2, 473 (1907).

[c] Compt. Rend., **141,** 1044 (1905).

[d] Am. Jour. Physiol., **14,** 299 (1905).

[e] Bul. 59, Hygienic Lab., Public Health and Marine-Hospital Service of the United States, 1910.

[f] Bul. Pharm., **2,** 578 (1810).

[g] Jour. Pharm., **5,** 565 (1819).

[h] Jour. Pharm., **6,** 16 (1820).

[i] Pogg. Ann., **50,** 616 (1840); **59,** 240 (1843).

[j] Pogg. Ann., **63,** 520 (1844).

the chemically excited or active condition in which it is met with in hydrogen peroxide, ozone, and manganese dioxide. From his study of the oxidation by active plant and animal tissues Schönbein [a] concluded that the tissues contained oxygen exciters and oxygen carriers or what are called to-day oxidizing enzymes. He recognized the value of the substances for the processes of oxidation occurring in the organism.

Traube [b] introduced the term oxidizing ferment in 1858. He developed the idea that ferments are in all probability chemical compounds originating from proteids and of a definite chemical composition. He concluded that various organic and inorganic substances other than those elaborated in the living cell may under certain conditions function as ferments.

With the investigations of Yoshida [c] and Bertrand [d] on the oxidative changes in the sap of *Rhus vernicifera*, the study of oxidation in plants and animals received a new and powerful stimulus. The changes in the fresh juice of the lac tree are brought about, according to Bertrand, by an oxidizing enzyme, laccase, which oxidizes polyphenols. Numerous other oxidases have been discovered, the best known of which is tyrosinase, which oxidizes tyrosine. In addition to the oxidases, two other classes of enzymes are more or less immediately concerned in the oxidative processes occurring in plants and animals. They are the peroxidases and catalases. The peroxidases oxidize substances in the presence of hydrogen peroxide. The catalases decompose hydrogen peroxide into water and molecular oxygen without being able to activate the oxygen of the peroxide toward oxidizable substances. The literature on the subject of plant and animal oxidation is exceedingly voluminous and can not be dealt with here. Suffice it to say, that within recent years it has been shown that many, if not all, of the oxidations, both direct and indirect, brought about by living tissues, can be duplicated by substances both organic and inorganic of known constitution. Whether or not the oxidizing substances of plant and animal tissue are true enzymes is somewhat debatable, and little is known as regards their chemical nature. Laccase has been studied most and has been found to be composed of both inorganic and organic material. The laccase of *Rhus vernicifera* Bertrand found to consist of an active part manganese and an activat-

[a] Pogg. Ann., **75,** 351 (1848); Verhandl. Naturf. Ges., Basel., **1,** 339 (1855); Jour. prakt. Chem., **67,** 496 (1855); Verhandl. Naturf. Ges., Basel., **1,** 467 (1857); Jour. prakt. Chem., **98,** 323 (1863).

[b] Theorie der Fermentwirkungen. Berlin. 1858. Pogg. Ann., **103,** 331 (1858).

[c] Jour. Chem. Soc., **43,** 472 (1883).

[d] Compte Rendu, **118,** 1215 (1894).

ing part which was organic. Oxidases which contain no manganese, but do contain other inorganic substances, have been reported by Sarthou,[a] Vadam,[b] and De Stoecklin.[c] The activating organic portion, which of itself has no oxidizing action, Bertrand believed he had found in other plants, such as *Medicago sativa*. The laccase of *Medicago sativa*, Euler and Bolin, as previously pointed out, found to consist of neutral salts, mainly calcium, of glycolic, mesoxalic, malic, and citric acids. Bach[d] recently reported on an active oxidase from molds which was entirely free from iron or manganese. Van der Haar,[e] however, doubts the validity of Bach's conclusion as regards the absence of manganese.

In the study of oxidation in soils we have found that most soils will directly oxidize substances in the manner of an oxidase, while a few will oxidize only in the presence of hydrogen peroxide, like the peroxidase reaction in plants and animals. The addition of certain organic hydroxyacids increases oxidation in soils in a way analogous to the activating action of alfalfa laccase and salts of hydroxyacids as discovered by Euler and Bolin. Dilute mineral acids and alkalies check oxidation in soils as they do the oxidation by plant roots or plant juices. The addition of various salts to soils increases the oxidizing power of the soil just as Bach[f] found that mineral salts further oxidative changes of tyrosine by tyrosinase. In short, it would be found undoubtedly that all the various kinds of oxidation going on in plants and animals could be duplicated by a detailed study of oxidation in soil. The oxidation in soil per se is due mainly to nonenzymotic forces, inorganic and organic, working separately, conjointly, or in reenforcing or activating combination. In soil oxidation the nature of the organic matter seems to be of great importance. Conjointly with the oxidation in soils, which is active whether the soil is planted or unplanted, is the oxidizing power of the roots.

The part that microorganisms play in decomposing organic matter which retards oxidation or in the formation of compounds of an activating nature or capable of acting as oxygen carriers must be of great importance, but can not be considered in detail here. Moreover, the consideration of the relation between microorganisms and oxidation in soil would require further experimental work of considerable scope.

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[a] Jour. Pharm., **11,** 482 (1900); **12,** 104 (1900); **13,** 464 (1901).
[b] Jour. Pharm., **9,** 515 (1899).
[c] Bot. Centr., **107,** 6 (1908).
[d] Ber. deutsch Chem. Ges., **43,** 364 (1910).
[e] Ber. deutsch Chem. Ges., **43,** 1321 (1910).
[f] Ber. deutsch Chem. Ges., **43,** 364 (1910).

SUMMARY.

Oxidations play an important part in the transformations, both mineral and organic, which go on in soils. In this bulletin are studied reduction and oxidation by roots, concurrent oxidation, and reduction by roots, and oxidation within the soil itself. The fact that roots possess the power of reduction was shown by the precipitation of tellurium and selenium from sodium tellurite and selenite, respectively. The oxidative power of the roots was shown by means of organic compounds, which, on oxidation, yield dyes which either color the solution or are deposited on the root surface. These two opposite properties may occur separately or concurrently, depending upon the reaction of the medium. The oxidation within the soil itself is shown by the same reagents as those showing oxidation by the roots. The reagent most successfully used in the case of soil is aloin, a yellow water solution of which is changed to a claret red by the oxidation. The depth of color can be measured and is taken as an indication of the extent of the oxidation.

This oxidation appears to be mainly nonenzymotic, the result of interaction between inorganic constituents and certain types of organic matter. It may also be brought about by organic matter in a state of autoxidation and by inorganic oxygen carriers, such as manganese and iron. Both processes activate oxygen.

The oxidation in soils was increased by the addition of salts of manganese, iron, aluminum, calcium, and magnesium, especially in the presence of simple hydroxyacids, such as citric, tartaric, malic, glycolic, and their salts. The best oxidation was obtained by the addition of manganese, and the stimulating action of manganese used as a fertilizer is attributed to its oxidizing power; i. e., to its amelioration of soil conditions rather than its function as a plant nutrient. Fertilizer salts augment the oxidizing power of roots, and the fertilized soil has an increased oxidizing power after cropping. The fertilizer salts alone sometimes increase, sometimes decrease, the oxidative functions of the soil itself, thus showing that the fertilizer salts are effecting changes directly or indirectly in the soil constituents, more particularly in the organic matter. Some types of organic matter, such as dihydroxystearic acid, isolated from certain soils, inhibit oxidation in the soil, but in the main the oxidative power is augmented by a plentiful supply of organic matter, the nature of which in the soil is the limiting factor of oxidation. Excessive oxidation is harmful to vegetation.

Oxidation in soils is parallel to oxidation in plants and animals, and all the various kinds of oxidation going on in these can undoubtedly be duplicated by a detailed study of oxidation in soils. The oxidation in soils per se is due mainly to nonenzymotic forces,

inorganic and organic, working separately, conjointly, or in reenforcing or activating combination. Soils oxidize substances in a manner analogous to an oxidase, and the increase noticed by the addition of certain hydroxyacids is closely paralleled by the recently discovered activating action of salts of tartaric and citric acids on the oxidative action of manganese acetate. This analogy between the oxidative power of a soil and the action of an oxidase is especially significant in that an oxidizing enzyme, laccase, of alfalfa, has been found to be more simple in composition than formerly supposed and to consist of neutral salts, mainly calcium, of glycolic, mesoxalic, malic, and citric, and probably glyoxylic acid.

It is well known that the oxidases, whatever may be their nature, play an important part in the proper functioning of plants, and that with change in oxidizing ability are associated changes in the plant conditions. Similarly, the oxidative power of the soil becomes, as it were, a symptom of soil condition.

Whatever decreases the oxidation in soils tends also to bring about the conditions which decrease growth, and the factors which favor oxidation are the factors which favor soil productivity.

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